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(71) Applicant (for all designated States except US): **NOVO  
NORDISK A/S** [DK/DK]; Novo Allé, DK-2880  
Bagsvaerd (DK).

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(72) Inventors; and

(75) Inventors/Applicants (for US only): **KNUDSEN, Jens**,  
**Bjerre** [DK/DK]; Enebovaenge 13, DK-3520 Farum  
(DK). **HEDNER, Ulla** [SE/SE]; Caritasgatan 19, S-21618  
Malmö (SE). **RØJKJÆR, Rasmus** [DK/DK]; Folkevej  
30A, DK-2820 Gentofte (DK).

(74) Common Representative: **NOVO NORDISK A/S**; Cor-  
porate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).

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(54) Title: COMBINED USE OF FACTOR VII POLYPEPTIDES AND FACTOR IX POLYPEPTIDES

(57) Abstract: The invention concerns a pharmaceutical preparation comprising a factor VII or factor VII-related polypeptide and a factor IX or factor IX-related polypeptide. The invention also concerns use of a factor VII or factor VII-related polypeptide and a factor IX or factor IX-related polypeptide for manufacture of a medicament for pharmaceutical use as well as methods for prevention or treatment of bleeding episodes in subjects.

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## Combined use of factor VII polypeptides and factor IX polypeptides

### FIELD OF INVENTION

5           The invention relates to a pharmaceutical composition comprising a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide. The invention also relates to a kit-of-parts for treatment of bleeding episodes comprising a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide. The invention also relates to use of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide for the preparation of a medicament. Furthermore, the invention relates to methods for treating bleedings, reducing clotting time, enhancing haemostasis, reducing the number of administrations of coagulation factor protein needed to accomplish haemostasis, reducing the amount of administered coagulation factor protein needed to accomplish haemostasis, prolonging clot lysis time, increasing clot strength, and enhancing fibrin clot formation.

### BACKGROUND OF INVENTION

Blood coagulation factor VII (FVII) is a plasma coagulation factor. Activated factor VII (FVIIa) initiates the normal haemostatic process by forming a complex with tissue factor (TF), exposed as a result of the injury to the vessel wall, which subsequently activates factors IX and X (FIX and FX) into their activated forms, factors IXa and Xa (FIXa and FXa). Factor Xa converts limited amounts of prothrombin to thrombin on the tissue factor-bearing cell. Thrombin activates platelets and factors V and VIII into factors Va and VIIIa (FVa and FVIIIa), both cofactors in the further process leading to the full thrombin burst. This process includes generation of factor Xa by factor IXa (in complex with factor VIIIa) and occurs on the surface of activated platelets. Thrombin finally converts fibrinogen to fibrin resulting in formation of a fibrin clot.

Factor VII exists in plasma mainly as a single-chain zymogen, which is cleaved by FXa into its two-chain, activated form, FVIIa. Recombinant activated factor VIIa (rFVIIa) has been developed as a pro-haemostatic agent. The administration of rFVIIa offers a rapid and highly effective pro-haemostatic response in haemophilic subjects with bleedings who cannot be treated with coagulation factor products due to antibody formation. Also bleeding subjects with factor VII deficiency or subjects having a normal coagulation system but experiencing excessive bleeding can be treated successfully with FVIIa. In these studies, no unfavourable side effects of rFVIIa (in particular the occurrence of thromboembolism) has been encountered.

35           Blood coagulation factor IX (factor IX) is a plasma coagulation factor participating in the activation of factor X (FX). A decrease in the presence or activity of Factor IX in the blood stream leads to haemophilia B. The level of the decrease in Factor IX activity is directly proportional to the severity of the disease. The current treatment of haemophilia B consists of the re-

placement of the missing protein by plasma-derived or recombinant factor IX (so-called FIX substitution or replacement treatment or therapy).

Coagulation factor deficiencies (e.g., FIX deficiency) reflect different types of gene defects. Where the genetic lesion is severe, such as, deletion or frame shift, mRNA is not produced and (severe) deficiency results. Less severe genetic lesions from, for instance, point mutations which are not critically located result in secretion of protein with reduced biological activity. The inheritance pattern is recessive and X-linked, meaning that only men having one X-chromosome are affected. The severity of the coagulation defect can be mild or severe. Severity depends on the concentration of normally functioning factor IX in plasma. The aim of replacement therapy is to raise the level of the patient's clotting factor activity (hereinafter called the "factor level") to one that will bring around haemostasis and to maintain it until healing is substantially complete. If the initiation of effective treatment is delayed, wound healing may be impaired and more treatment than usual will be required. The amount of treatment depends upon the plasma concentration of the coagulation factor needed for haemostasis, the recovery in blood and the half-life of the transfused material.

The level of factor IX may also be more or less reduced in some subjects (e.g., women being carriers of the disease) who are heterozygous for the gene defect. Such subjects may have an increased bleeding tendency comparable to that of a mildly-affected haemophiliac and may be treated accordingly.

Some patients receiving factor IX replacement therapy (having haemophilia B) develop antibodies against the administered factor IX. However, persons born with a normal factor IX level (not having a congenital factor IX-deficiency) may for unknown reasons later in life develop auto-antibodies against factor IX (acquired haemophilia B). In both cases the antibodies may be present in low, medium or high titres. In case of patients having a low or medium inhibitor-titre, these may sometimes be treated with factor IX.

Haemophilia occurs in all degrees of severity. The patient with no detectable or less than 1% factor IX is usually severely affected and bleeds into muscles and joints on minimal trauma and sometimes apparently spontaneously. A small amount of factor IX gives considerable protection so that patients with 1-5% of normal level factor IX usually suffer only posttraumatic bleeding and less severe bleeding into muscles and joints, etc., and are often said to be moderately affected. Patients with more than 5% of factor IX usually bleed only after significant trauma or surgery and are said to be mildly affected. It must be realised that this classification is not always valid in individual cases. Some patients with very low factor IX levels rarely bleed whilst others even with over 5% factor IX may bleed repeatedly into the "target joint" damaged originally by a traumatic haemarthrosis and appear to be "severely" affected. As a generalisation, however, bleeding symptoms are less obvious with higher factor levels so that abnormal bleeding does not usually occur at factor IX levels over 35-40% of normal level. The general correlation between factor levels and symptoms in haemophilia B is shown below.

## Severity of haemophilia related to factor IX levels:

Severity	Factor Level (% of normal level)	Type of presentation
Severe	0-1	Apparently spontaneous bleeds. Severe bleeding
Moderate	1-5	Few bleeds. Haemarthroses mainly traumatic
Mild	5-30	Post-traumatic, post-surgical, post-dental extraction bleeding. Few episodes.

5 The current treatment of haemophilia B consists of the replacement of the missing protein by plasma-derived or recombinant factor IX. Factor IX products are used as I.V. infusion (or injection) to treat acute bleeds on demand. The bleeding types are categorised as follows:

1. Haemarthrosis (bleeding in joints)
2. Life-and limb threatening bleeds (retroperitoneal bleeds, CNS bleeds, retropharyngeal bleeds, muscular bleeds with compartment syndrome and massive GI bleeds)
- 10 3. Bleeding prevention in relation to surgery (orthopaedic, elective procedures, emergency surgery)

Experience has shown that if factor IX levels are maintained over 30-40% of normal level until healing is complete then normal haemostasis is usually maintained. However other considerations are also important. Movement of the affected parts such as a haemarthrosis, coughing or walking after abdominal surgery may promote bleeding. Physiotherapy or manipulation may require rather high levels whilst immobilisation of mild lesions may allow control of bleeding with relatively low factor levels. Approximate target levels which can be aimed for in various situations are shown below:

20 *Treatment of standard haemarthrosis (category 1):*

The normal intent is to achieve an initial factor IX plasma concentration of at least 20 – 30 % of normal level followed by a plasma concentration of at least 10 - 20 % of normal level for 2 – 3 days.

*Treatment of Life-and limb threatening bleeds (category 2):*

25 The normal intent is to achieve an initial factor IX plasma concentration of at least 40 % followed by a plasma concentration of at least 10 - 20 % for one week.

*Bleeding prevention in relation to surgery (category 3):*

The normal intent is to achieve a factor IX plasma concentration of at least 55 – 80 % on the day of surgery followed by a plasma concentration of at least 20 – 30 % from day 2 to 7 and continuing with a plasma concentration of at least 10 - 20 % for one to two weeks.

30 Following the above guidelines for treatment, the following can be said of the number of factor IX injections in relation to types of bleedings.

With an average plasma half-life of factor IX of 18-24 hours the following average numbers of injections of factor IX per bleeding episode are normally used in clinical praxis:

*Haemarthrosis (bleeding in joints):* Home treatment, minor haemarthrosis: 1-3 injections; Hospital treatment, larger haemarthrosis: 6 – 14 injections.

5 *Life-and limb threatening bleeds:* 10 – 20 injections.

*Bleeding prevention in relation to surgery:* 30 – 40 injections

In clinical treatment of haemophilia FVIIa is presently used to stop bleedings in patients having inhibitors to FVIII or FIX (which prevents replacement therapy). However, clinicians do not  
10 normally use FVIIa as first line treatment for haemophiliacs without inhibitors (where FVIII or FIX, respectively, can be used) because it is expected that the short half-life of factor VIIa compared to that of factor IX (2.5 hours compared to 18-24 hours) would require more frequent factor VIIa injections to maintaining a certain level of haemostatic ability.

European Patent No. 225.160 (Novo Nordisk) concerns compositions of FVIIa and meth-  
15 ods for the treatment of bleeding disorders not caused by clotting factor defects or clotting factor inhibitors.

European Patent No. 82.182 (Baxter Travenol Lab.) concerns a composition of factor VIIa for use in counteracting deficiencies of blood clotting factors or the effects of inhibitors to blood clotting factors in a subject.

20 Lusher et al., Haemophilia, 1998, 4, pp.790-798 concerns the administration of recombinant factor VIIa in treatment of joint, muscle and mucotaneous haemorrhages in persons with haemophilia A and B, with and without inhibitors.

Kjalke et al, Thrombosis and Haemostasis, 1999 (Suppl), 095 1 concerns the administration of extra exogenous FVIIa and the effect on the formation of thrombin on the activated  
25 platelet surface in a model system mimicking haemophilia A or B conditions.

U.S. Patent No. 5,891,843 (Immuno) concerns a composition of FVIIa in combination with a second ingredient having FEIB-activity, e.g., activated prothrombin complex or a FEIBA preparation.

Today, many factor IX products used in treatment of haemophilia contain recombi-  
30 nantly produced factor IX. However, the products may also have been isolated from human or porcine plasma. These purified products often contain lesser amounts of other coagulation factors or other components from plasma. Normally, such additional plasma components are unwanted (due to risk of viral infection or other contamination), and the replacement of part of such products with a recombinant protein (e.g., factor VIIa) will be considered an improvement  
35 of the composition and treatment and a benefit to the patient.

Today, subjects having a reduced level of factor IX (e.g., haemophilia B patients) experiencing bleeding episodes are generally treated with several injections, or infusions, of factor

IX before the bleeding is stopped. Furthermore, a considerable number of injections are needed to maintain haemostasis until the injury causing the bleeding is completely healed.

Trauma victims, suffering from excessive bleedings, are generally treated with large infusion volumes of fluids, such as fluids for intra venous (i.v.), injection colloid infusion products, albumin, red blood cell concentrates, etc. Extensive bleedings requiring massive blood transfusions may lead to the development of multiple organ failure including impaired lung and kidney function.

A faster arrest of bleedings would be an important benefit to such subjects. So would a reduction in the number of injections needed to stop bleeding and maintain haemostasis and or a reduction in the amount of coagulation protein usage for bleeding arrest and maintaining haemostasis.

There is still a need in the art for improved treatment of subjects experiencing bleeding episodes, including subjects where the bleeding episodes are due to a reduced level of coagulation factor IX. There remains a need in the art for improved, reliable and widely applicable methods of enhancing coagulation, enhancing or ensuring formation of stable haemostatic plugs, enhancing convenience for the treated subject, or achieving full or sufficient haemostasis in subjects, in particular in subjects having an impaired thrombin generation. There is also a need for methods wherein the amount of FVIIa or the amount of FIX needed for achieving full or sufficient haemostasis is lowered. There is also a need for methods wherein the total amount of coagulation factor protein needed for achieving full or sufficient haemostasis is lowered and methods wherein the time to bleeding arrest is shortened

#### SUMMARY OF THE INVENTION

One object of the present invention is to provide compositions, which can effectively be used in the treatment or prophylaxis of bleeding episodes and coagulation disorders.

A second object of the present invention is to provide compositions in one dosage form, which can effectively be used in the treatment or prophylaxis of bleeding episodes or as a procoagulant. Another object of the present invention is to provide compositions, methods of treatment or kits exhibiting a synergistic effect.

A further object of the present invention is to provide compositions, methods of treatment or kits exhibiting no substantial side effects, such as a high level of systemic activation of the coagulation system.

Other objects of the present invention will become apparent upon reading the present description.

In a first aspect the invention concerns a pharmaceutical composition comprising a preparation of a factor VII or a factor VII-related polypeptide, and a preparation of a factor IX or a factor IX-related polypeptide.

In a second aspect the invention concerns a kit-of-parts containing a treatment for bleeding episodes comprising a) An effective amount of a preparation of a factor VII or factor VII-related polypeptide and a pharmaceutically acceptable carrier in a first unit dosage form; b) An effective amount of a preparation of a factor IX or factor IX-related polypeptide and a pharmaceutically acceptable carrier in a second unit dosage form; and c) Container means for containing said first and second dosage forms.

In another aspect the invention concerns the use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or a factor IX-related polypeptide for the manufacture of a medicament for treating bleeding episodes in a subject.

In another aspect the invention concerns the use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for reducing clotting time

In another aspect the invention concerns the use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for prolonging the clot lysis time.

In another aspect the invention concerns the use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for increasing clot strength.

In another aspect the invention concerns a method for treating bleeding episodes in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amount together are effective to treat bleedings.

In another aspect the invention concerns a method for reducing clotting time in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to reduce clotting time.

In another aspect the invention concerns a method to enhance haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to enhance haemostasis.

In another aspect the invention concerns a method for reducing the number of administrations of coagulation factor protein needed to arrest bleeding and maintain haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a prepara-

tion of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to arrest bleeding and maintain haemostasis.

In another aspect the invention concerns a method for reducing the amount of administered coagulation factor protein needed to arrest bleeding and maintain haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to arrest bleeding and maintain haemostasis.

In another aspect the invention concerns method for prolonging the clot lysis time in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to prolong the clot lysis time.

In another aspect the invention concerns a method for increasing clot strength in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to increase clot strength.

In another aspect the invention concerns a method for enhancing fibrin clot formation in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to enhance fibrin clot formation.

In another aspect the invention concerns a kit-of-parts containing a treatment for bleeding episodes comprising a) An effective amount of a factor VII or factor VII-related polypeptide and an effective amount of a factor IX or factor IX-related polypeptide and a pharmaceutically acceptable carrier in a one-unit dosage form; and b) Container means for containing said one-unit dosage form.

In one series of embodiments of the invention, the factor VII or factor VII-related polypeptide is a factor VII-related polypeptide. In another embodiment the factor VII or factor VII-related polypeptide is a factor VII polypeptide. In one series of embodiments of the invention said factor VII-related polypeptide is a factor VII amino acid sequence variant. In one embodiment the ratio between the activity of the factor VII-related polypeptide and the activity of native human factor VIIa (wild-type FVIIa) is at least about 1.25 when tested in the "In Vitro Hydrolysis Assay" as described in the present description.

In one series of embodiments of the invention the factor VII or factor VII-related polypeptide is a factor VII polypeptide. In one embodiment the factor VII is human factor VII. In one embodiment the factor VII is bovine, porcine, canine, equine, murine or salmon factor VII. In



another embodiment the factor VII polypeptide is recombinant factor VII. In another embodiment the factor VII polypeptide is plasma-derived factor VII. In another embodiment the factor VII polypeptide is plasma-derived human factor VII. In another embodiment the factor VII polypeptide is recombinant human factor VII. In one series of embodiments of the invention the factor VII or factor VII-related polypeptide is in its activated form. In one embodiment of the invention the factor VII polypeptide is recombinant human factor VIIa.

In one series of embodiments the factor IX or factor IX-related polypeptide is a factor IX-related polypeptide. In one embodiment the factor IX-related polypeptide is a factor IX amino acid sequence variant. In one embodiment the ratio between the activity of the factor IX-related polypeptide and the activity of native human factor IX (wild-type FIX) is at least about 1.25 when tested in the "chromonic assay" as described in the present description. In one embodiment the factor IX or factor IX-related polypeptide is a factor IX polypeptide. In one embodiment the factor IX is human factor IX. In one embodiment the factor IX is bovine, porcine, canine, equine, murine or salmon factor IX. In another embodiment the factor IX polypeptide is recombinant factor IX. In another embodiment the factor IX polypeptide is plasma-derived factor IX. In another embodiment the factor IX polypeptide is plasma-derived human factor IX. In another embodiment the factor IX polypeptide is recombinant human factor IX. In one series of embodiments of the invention the factor IX or factor IX-related polypeptide is in its activated form

In one embodiment the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are present in a ratio by mass of between about 100:1 and about 1:100 factor VII:factor IX.

In one embodiment, the factor VII-related polypeptides are amino acid sequence variants having no more than 20 amino acids replaced, deleted or inserted compared to wild-type factor VII (i.e., a polypeptide having the amino acid sequence disclosed in U.S. Patent No. 4,784,950). In another embodiment, the factor VIIa variants have no more than 15 amino acids replaced, deleted or inserted; in another embodiment, the factor VII variants have no more than 10 amino acids replaced, deleted or inserted; in another embodiment, the factor VII variants have no more than 8 amino acids replaced, deleted or inserted; in another embodiment, the factor VII variants have no more than 6 amino acids replaced, deleted or inserted; in another embodiment, the factor VII variants have no more than 5 amino acids replaced, deleted or inserted; in another embodiment, the factor VII variants have no more than 3 amino acids replaced, deleted or inserted compared to wild-type factor VII. In one embodiment, the factor VII variants are selected from the list of L305V-FVIIa, L305V/M306D/D309S-FVIIa, L305I-FVIIa, L305T-FVIIa, F374P-FVIIa, V158T/M298Q-FVIIa, V158D/E296V/M298Q-FVIIa, K337A-FVIIa, M298Q-FVIIa, V158D/M298Q-FVIIa, L305V/K337A-FVIIa, V158D/E296V/M298Q/L305V-FVIIa, V158D/E296V/M298Q/K337A-FVIIa, V158D/E296V/M298Q/L305V/K337A-FVIIa, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII

In a further aspect, the factor VII or factor VII-related polypeptides have increased tissue factor-independent activity compared to native human coagulation factor VIIa. In another aspect, the increased activity is not accompanied by changes in the substrate specificity. In another aspect of the invention, the binding of the factor VII or factor VII-related polypeptides to tissue factor should not be impaired and the factor VII or factor VII-related polypeptides should have at least the activity of wild-type factor VIIa when bound to tissue factor.

In one preferred embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are recombinant human factor VIIa and recombinant human factor IX.

In one embodiment, the clotting time is reduced in mammalian blood. In another embodiment the haemostasis is enhanced in mammalian blood. In another embodiment the clot lysis time is prolonged in mammalian blood. In another embodiment the clot strength is increased in mammalian blood. In another embodiment the fibrin clot formation is enhanced in mammalian blood. In one embodiment, the mammalian blood is human blood. In another embodiment, the mammalian blood is normal blood; in another embodiment, the mammalian blood is blood having a normal level of coagulation factor proteins; in another embodiment, the mammalian blood is blood having a normal level of factor IX; in another embodiment, the blood is normal human blood; in one embodiment, the blood is blood from a subject having an impaired thrombin generation. In one embodiment, the blood is blood from a subject having a deficiency of one or more coagulation factors; in another embodiment, the blood is blood from a subject having inhibitors against one or more coagulation factors. In one embodiment, the blood is from a subject having a lowered concentration of fibrinogen. In one embodiment, the blood is factor IX-deficient human blood.

In one embodiment of the invention, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are the sole haemostatic agents employed. In another embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are the sole active haemostatic agents employed. In another embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are the sole coagulation factors employed. In one embodiment of the invention, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are the sole active agents employed. "Sole" agents or factors as used herein refers to situations in which the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide, taken together, are the only haemostatic agents, active haemostatic agents, or coagulation factors, as applicable, contained in the pharmaceutical composition or kit, or are the only haemostatic agents, active haemostatic agents, or coagulation factors, as applicable, administered to the patient in the course of a particular treatment, such as, e.g., in the course of a particular bleeding episode. It will be understood that these situations encompass those in which other

haemostatic agents or coagulation factors, as applicable, are not present in either sufficient quantity or activity so as to significantly influence one or more coagulation parameters.

In another embodiment, the pharmaceutical composition is formulated for intravenous administration. In one embodiment, the composition further contains a pharmaceutical acceptable  
5 excipient.

In one embodiment of the invention, the composition is in single-dosage form wherein the single-dosage form contains both coagulation factors. In one embodiment of the invention, the composition is in the form of a kit-of-parts comprising a preparation of a factor VII or factor VII-related polypeptide as a first unit dosage form and a preparation of a factor IX or factor IX-  
10 related polypeptide as a second unit dosage form, and comprising container means for containing said first and second dosage forms. In one embodiment the composition or kit, as applicable, further contains directions for the administration of the composition or separate components, respectively.

In one embodiment of the invention, the factor VII or factor VII-related polypeptide  
15 and the factor IX or factor IX-related polypeptide are administered in single-dosage form. In one embodiment of the invention, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered in the form of a first unit dosage form comprising a preparation of a factor VII or factor VII-related polypeptide and a second unit dosage form comprising a preparation of a factor IX or factor IX-related polypeptide.

In one embodiment of the invention, the factor VII or factor VII-related polypeptide  
20 and the factor IX or factor IX-related polypeptide are administered simultaneously. In another embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered sequentially. In one embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered with  
25 a time separation of no more than 15 minutes, preferably 10, more preferred 5, more preferred 2 minutes. In one embodiment, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered with a time separation of up to 2 hours, preferably from 1 to 2 hours, more preferred up to 1 hour, more preferred from 30 minutes to 1 hour, more preferred up to 30 minutes, more preferred from 15 to 30 minutes.

In one embodiment, the effective amount of the factor VII or factor VII-related  
30 polypeptide is an amount from about 0.05 mg/day to about 500 mg/day (70-kg subject). In one embodiment, the effective amount of a preparation of a factor IX or factor IX-related polypeptide is from about 0.01 mg/day to about 500 mg/day (70-kg subject).

In one embodiment the factor VII or factor VII-related polypeptide and factor IX or  
35 factor IX-related polypeptide are present in a ratio by mass of between about 100:1 and about 1:100 factor VII:factor IX.

In one embodiment of the present invention, the pharmaceutical composition is in single-dosage form and consists essentially of a preparation of a factor VII or factor VII-related

polypeptide and a preparation of a factor IX or factor IX-related polypeptide, and one or more of the components selected from the list of pharmaceutical acceptable excipients or carriers, stabilizers, detergents, neutral salts, antioxidants, preservatives, and protease inhibitors.

In a further embodiment, the subject is a human; in another embodiment, the subject  
5 has an impaired thrombin generation; in one embodiment, the subject has a lowered plasma concentration of fibrinogen (e.g., a multi-transfused subject); in one embodiment, the subject has a lowered plasma concentration of factor IX.

In one embodiment, the pharmaceutical composition is for home treatment

In another aspect, the invention concerns the use of a preparation of a factor VII or fac-  
10 tor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide for the preparation of a medicament for the treatment of bleedings in a subject suffering from a factor IX-responsive syndrome.

In another aspect, the invention concerns the use of a preparation of a factor VII or fac-  
tor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide for  
15 the preparation of a medicament for the treatment of bleedings in a subject having a reduced level of factor IX.

In another aspect, the invention concerns a method to enhance haemostasis in a subject  
suffering from a factor IX responsive syndrome compared to when the subject is treated with  
factor IX as the only coagulation protein, the method comprising administering to the subject in  
20 need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to enhance haemostasis.

In another aspect, the invention concerns a method to enhance haemostasis in a subject  
having a reduced level of factor IX compared to when the subject is treated with factor IX as the  
25 only coagulation protein, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to enhance haemostasis.

In another aspect, the invention concerns a method to enhance formation of thrombin in  
30 a subject, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to enhance formation of thrombin.

In another aspect, the invention concerns a method to enhance formation of thrombin in  
35 a subject suffering from a factor IX responsive syndrome compared to when the subject is treated with factor IX as the only coagulation protein, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypep-

tide, wherein the first and second amounts together are effective to enhance formation of thrombin.

In another aspect, the invention concerns a method to enhance formation of thrombin in a subject having a reduced level of factor IX compared to when the subject is treated with factor IX as the only coagulation protein, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to enhance formation of thrombin.

In another aspect, the invention concerns a method for reducing the number of administrations of coagulation factor protein needed to accomplish haemostasis in a subject suffering from a factor IX responsive syndrome compared to the number of administrations needed when factor IX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to reduce the number of administrations of coagulation factor protein.

In another aspect, the invention concerns a method for reducing the number of administrations of coagulation factor protein needed to accomplish haemostasis in a subject having a reduced level of factor IX compared to the number of administrations needed when factor IX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to reduce the number of administrations of coagulation factor protein.

In another aspect, the invention concerns a method for reducing the amount of administered coagulation factor protein needed to accomplish haemostasis in a subject suffering from a factor IX responsive syndrome compared to the amount of administered coagulation factor protein needed when factor IX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective to reduce the amount of administered coagulation factor protein.

In another aspect, the invention concerns a method for reducing the amount of administered coagulation factor protein needed to accomplish haemostasis in a subject having a reduced level of factor IX compared to the amount of administered coagulation factor protein needed when factor IX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or fac-

tor IX-related polypeptide, wherein the first and second amounts together are effective to reduce the amount of administered coagulation factor protein.

In another aspect, the invention concerns a method of treating bleedings in a subject suffering from a factor IX responsive syndrome, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective in treating bleedings.

In another aspect, the invention concerns a method of treating bleedings in a subject having a reduced level of factor IX, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective in treating bleedings.

In one embodiment, the subject has a reduced level of factor IX. In one embodiment the subject suffers from a factor IX-responsive syndrome. In one embodiment the factor IX responsive syndrome is haemophilia B.

In one embodiment the reduced factor IX level is 90% of normal level or below, in another embodiment the factor IX level is 80% or below, in another embodiment 50% or below, in another embodiment 40% or below, in another embodiment 30% or below, in another embodiment 20% or below, in another embodiment 10% or below, in another embodiment 5% or below, in another embodiment 2% or below. The terms may, where appropriate, be used interchangeably. In a preferred embodiment, the factor IX level is below 30 % of normal level.

In another aspect, the invention concerns a method of treating bleedings in a subject suffering from a factor IX responsive syndrome, the method comprising administering to the subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amounts together are effective in treating bleedings.

In one embodiment, the factor VII is human recombinant factor VIIa (rFVIIa). In another embodiment, the rFVIIa is NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark).

In one embodiment, the factor IX is human recombinant factor IX (rFIX). In another embodiment, the factor IX product is BeneFix (AHP/Genetics Institute), Mononine (Aventis), Proplex (Baxter), Bebulin VH (Baxter), FACTEUR IX-LFB (Laboratoire Francais du Fractionnement et des Biotechnologies (LFB)), Immunine (Baxter/Immuno), Octanyne (Octapharma), Octanine F (Octapharma), Mono FIX-VF (CSL), Novact M (Kaketsuken).

In one embodiment, the pharmaceutical composition is formulated for intravenous administration. In one embodiment, the composition further comprises an inhibitor of the fibrinolytic system, including, without limitation, aprotinin,  $\epsilon$ -aminocaproic acid or tranexamic acid.

LIST OF FIGURES

Figure 1: The clot shortening effect of rFVIIa, in the absence and presence of FIX is shown in figure 1.

#### DETAILED DESCRIPTION OF INVENTION

5           Subjects, who bleed excessively in association with surgery or major trauma thus needing blood transfusions, develop more complications than those who do not experience any bleeding. However, also moderate bleedings may lead to complications if they require the administration of human blood or blood products (platelets, leukocytes, plasma-derived concentrates for the treatment of coagulation defects, etc.) because this is associated with the risk of  
10           transferring human viruses (e.g., hepatitis, HIV, parvovirus, or other, by now unknown viruses) as well as non-viral pathogens. Extensive bleedings requiring massive blood transfusions may lead to the development of multiple organ failure including impaired lung and kidney function. Once a subject has developed these serious complications a cascade of events involving a number of cytokines and inflammatory reactions is started making any treatment extremely difficult and  
15           unfortunately often unsuccessful. Therefore, a major goal in surgery as well as in the treatment of major tissue damage is to avoid or minimise the bleeding. To avoid or minimize such unwanted bleedings it is important to ensure formation of stable and solid haemostatic plugs that are not readily dissolved by fibrinolytic enzymes. Furthermore, it is of importance to ensure quick and effective formation of such plugs or clots.

20           Subjects with thrombocytopenia (lowered count or activity of platelets) also have an impaired thrombin generation as well as a defective stabilization of the fibrin plugs resulting in haemostatic plugs prone to premature dissolution. Furthermore, subjects subjected to major trauma or organ damage and who, as a consequence, have obtained frequent blood transfusions often have lowered platelet counts as well as lowered levels of fibrinogen, factor VIII, and other  
25           coagulation proteins. These subjects experience an impaired (or lowered) thrombin generation. These subjects, therefore, have a defective, or less efficient, haemostasis leading to the formation of fibrin plugs that are easily and prematurely dissolved by proteolytic enzymes, such enzymes in addition being extensively released in situations characterized by extensive trauma and organ damage.

30           A patient experiencing a major loss of blood becomes clinically unstable. Such patient are in risk of experiencing auricular fibrillation, which may lead to a fatal stop of cardiac activity; impaired renal function; or fluid extravasations in lungs (so-called "wet lungs" or ARDS).

            Bleedings in tissues may also lead to the formation of haematomas. The sizes of (in particular intracranial and spinal) haematomas are closely correlated to the extent of loss of neurological function, rehabilitation difficulties, and/or the severity and degree of permanent impairments of neurological function following rehabilitation. The most severe consequences of  
35           haematomas are seen when they are located in the brain where they may even lead to the death of the patient. The so-called compartment syndrome is a clinical condition caused by heavy

bleeding internally into an extremity. In arms and legs the muscles and bones are externally confined by an almost inelastic collagen sheet called the fascia. Bleeding in spaced confined by the fascia will lead to increased pressure in that compartment and subsequent pressure to nerves, vessels and muscle tissues, thus causing extensive tissue necrosis if not treated immediately. If

5 formed, necrotic tissue will to a large extent, during the event of healing, be transformed into connective tissue, which is contracted compared to the original muscle tissue. Such contractures make the subject liable to experience impaired motility of affected joints which again leads to the need of corrective surgery. Severe haematomas may furthermore lead to formation of pseudo cysts which may be likened to benign tumours in that such cysts, like tumours, erode the  
10 affected muscle or bone tissues. Again, surgery is needed to remove such pseudo cysts.

Formation of haematomas furthermore increases the frequency of infections in a subject. So does infusion of blood products such as, e.g., red blood cells. Infusions of red blood cells lead to a risk of formation of antibodies in the subject. When antibodies to blood type antigens have been formed transfusion of the subject are difficult as it will be increasingly difficult to find  
15 suitable types of blood.

Thus, major objectives in treatment of bleedings are to obtain haemostasis in a minimum of time, thus keeping the blood loss at a minimum.

The present invention thus provides beneficial compositions, uses and methods of treatment for treatment of bleeding episodes in subjects in need of such treatment. The compositions, uses and methods may be associated with beneficial effects such as less blood loss before  
20 haemostasis is obtained, less blood needed during surgery, blood pressure kept at an acceptable level until haemostasis is obtained, faster stabilisation of blood pressure, shorter recovery time for the treated patient, shorter rehabilitation time for the treated patient, diminished formation of haematomas or formation of smaller haematomas, including haematomas in the brain, less  
25 formation of pseudo cysts, less formation of muscle contractures, faster arrest of bleedings, reduction in the number of injections needed to stop bleeding and maintain haemostasis, reduction in the amount of coagulation protein usage for arresting bleeding and maintaining haemostasis.

The administration of a preparation of a factor VII or factor VII-related polypeptide, e.g., factor VIIa, in combination with a preparation of a factor IX or factor IX-related polypeptide  
30 provides a shortened clotting time compared to the clotting time when either factor VIIa or factor IX is administered alone.

The administration of a preparation of a factor VII or factor VII-related polypeptide, e.g., factor VIIa, in combination with a preparation of a factor IX or factor IX-related polypeptide  
35 also provides for a reduced total amount of coagulation factor usage to arrest bleeding and maintain haemostasis in a subject in need of such treatment compared to the protein usage when either factor VIIa or factor IX is administered alone.



The administration of a preparation of a factor VII or factor VII-related polypeptide, e.g., factor VIIa, in combination with a preparation of a factor IX or factor IX-related polypeptide also provides for a reduced time to obtain bleeding arrest and a reduced number of injections to maintain haemostasis compared to the situation when either factor VIIa or factor IX is administered alone. The administration of a preparation of a factor VII or factor VII-related polypeptide, e.g., factor VIIa, in combination with a preparation of a factor IX or factor IX-related polypeptide will also provide for a reduced number of injections to ensure full haemostasis compared to the situation when either factor IX or factor VIIa is administered alone.

In patients suffering from haemophilia B the present invention provides a beneficial effect of simultaneous or sequential dosing of a preparation of a factor IX or factor IX-related polypeptide and a preparation of a factor VII or factor VII-related polypeptide. Coagulation factor IX substitution and a preparation of a factor VII or factor VII-related polypeptide both induce arrest of bleeding in these patient groups, but have different biochemical mechanisms of action. Simultaneous dosing increases the haemostatic effect.

The present invention provides a pharmaceutical composition comprising a combination of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide. The composition may be in the form of a single composition or it may be in the form of a multi-component kit (kit-of-parts). The composition according to the present invention is useful as a therapeutic and prophylactic procoagulant in mammals, including primates such as humans. The present invention further provides a method for treating (including prophylactically treating or preventing) bleeding episodes in a subject, including a human being.

Whenever, a first or second or third, etc., unit dose is mentioned throughout this specification this does not indicate the preferred order of administration, but is merely done for convenience purposes.

A combination of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is an advantageous product ensuring short clotting times and rapid formation of haemostatic plugs. The present inventors will show that a combination of factor VIIa and factor IX can shorten the clotting time of normal human plasma more effectively than factor VIIa or factor IX alone. Thus, by shortening the clotting time a more effective treatment of bleedings in subjects can be obtained. Moreover, patients may be treated with lower total amounts of factor VII and factor IX or factor IX-related polypeptides.

#### Factor VII Polypeptides:

In practicing the present invention, any factor VII polypeptide may be used that is effective in preventing or treating bleeding. This includes factor VII polypeptides derived from blood or plasma, or produced by recombinant means.

The present invention encompasses factor VII polypeptides, such as, e.g., those having the amino acid sequence disclosed in U.S. Patent No. 4,784,950 (wild-type human factor VII). In some embodiments, the factor VII polypeptide is human factor VIIa, as disclosed, e.g., in U.S. Patent No. 4,784,950 (wild-type factor VII). In one series of embodiments, factor VII polypeptides  
5 include polypeptides that exhibit at least about 10%, preferably at least about 30%, more preferably at least about 50%, and most preferably at least about 70%, of the specific biological activity of human factor VIIa. In one series of embodiments, factor VII polypeptides include polypeptides that exhibit at least about 90%, preferably at least about 100%, preferably at least about 120%, more preferably at least about 140%, and most preferably at least about 160%, of  
10 the specific biological activity of human factor VIIa. In one series of embodiments, factor VII polypeptides include polypeptides that exhibit at least about 70 %, preferably at least about 80 %, more preferably at least about 90 %, and most preferable at least about 95 %, of identity with the sequence of wild-type factor VII as disclosed in U.S. Patent No. 4,784,950.

As used herein, "factor VII polypeptide" encompasses, without limitation, factor VII, as  
15 well as factor VII-related polypeptides. The term "factor VII" is intended to encompass, without limitation, polypeptides having the amino acid sequence 1-406 of wild-type human factor VII (as disclosed in U.S. Patent No. 4,784,950), as well as wild-type factor VII derived from other species, such as, e.g., bovine, porcine, canine, murine, and salmon factor VII, said factor VII derived from blood or plasma, or produced by recombinant means. It further encompasses natural allelic  
20 variations of factor VII that may exist and occur from one individual to another. Also, degree and location of glycosylation or other post-translation modifications may vary depending on the chosen host cells and the nature of the host cellular environment. The term "Factor VII" is also intended to encompass Factor VII polypeptides in their uncleaved (zymogen) form, as well as those that have been proteolytically processed to yield their respective bioactive forms, which  
25 may be designated Factor VIIa. Typically, Factor VII is cleaved between residues 152 and 153 to yield Factor VIIa.

"Factor VII-related polypeptides" include, without limitation, factor VII polypeptides that have either been chemically modified relative to human factor VII and/or contain one or more amino acid sequence alterations relative to human factor VII (i.e., factor VII variants),  
30 and/or contain truncated amino acid sequences relative to human factor VII (i.e., factor VII fragments). Such factor VII-related polypeptides may exhibit different properties relative to human factor VII, including stability, phospholipid binding, altered specific activity, and the like. The term "factor VII-related polypeptides" are intended to encompass such polypeptides in their uncleaved (zymogen) form, as well as those that have been proteolytically processed to yield  
35 their respective bioactive forms, which may be designated "factor VIIa-related polypeptides" or "activated factor VII-related polypeptides"

As used herein, "factor VII-related polypeptides" encompasses, without limitation, polypeptides exhibiting substantially the same or improved biological activity relative to wild-

type human factor VII, as well as polypeptides in which the factor VIIa biological activity has been substantially modified or reduced relative to the activity of wild-type human factor VIIa. These polypeptides include, without limitation, factor VII or factor VIIa that has been chemically modified and factor VII variants into which specific amino acid sequence alterations have been introduced that modify or disrupt the bioactivity of the polypeptide.

It further encompasses polypeptides with a slightly modified amino acid sequence, for instance, polypeptides having a modified N-terminal end including N-terminal amino acid deletions or additions, and/or polypeptides that have been chemically modified relative to human factor VIIa.

Factor VII-related polypeptides, including variants of factor VII, whether exhibiting substantially the same or better bioactivity than wild-type factor VII, or, alternatively, exhibiting substantially modified or reduced bioactivity relative to wild-type factor VII, include, without limitation, polypeptides having an amino acid sequence that differs from the sequence of wild-type factor VII by insertion, deletion, or substitution of one or more amino acids.

Factor VII-related polypeptides, including variants, encompass those that exhibit at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, or at least about 130%, of the specific activity of wild-type factor VIIa that has been produced in the same cell type, when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described above.

Factor VII-related polypeptides, including variants, having substantially the same or improved biological activity relative to wild-type factor VIIa encompass those that exhibit at least about 25%, preferably at least about 50%, more preferably at least about 75%, more preferably at least about 100%, more preferably at least about 110%, more preferably at least about 120%, and most preferably at least about 130% of the specific activity of wild-type factor VIIa that has been produced in the same cell type, when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described above.

Factor VII-related polypeptides, including variants, having substantially reduced biological activity relative to wild-type factor VIIa are those that exhibit less than about 25%, preferably less than about 10%, more preferably less than about 5% and most preferably less than about 1% of the specific activity of wild-type factor VIIa that has been produced in the same cell type when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described above. Factor VII variants having a substantially modified biological activity relative to wild-type factor VII include, without limitation, factor VII variants that exhibit TF-independent Factor X proteolytic activity and those that bind TF but do not cleave Factor X.

In some embodiments the factor VII polypeptides are factor VII-related polypeptides, in particular variants, wherein the ratio between the activity of said factor VII polypeptide and the

activity of native human factor VIIa (wild-type FVIIa) is at least about 1.25 when tested in the "In Vitro Hydrolysis Assay" (see "Assays", below); in other embodiments, the ratio is at least about 2.0; in further embodiments, the ratio is at least about 4.0. In some embodiments of the invention, the factor VII polypeptides are factor VII-related polypeptides, in particular variants, wherein the ratio between the activity of said factor VII polypeptide and the activity of native human factor VIIa (wild-type FVIIa) is at least about 1.25 when tested in the "In Vitro Proteolysis Assay" (see "Assays", below); in other embodiments, the ratio is at least about 2.0; in further embodiments, the ratio is at least about 4.0; in further embodiments, the ratio is at least about 8.0.

In some embodiments, the factor VII polypeptide is human factor VII, as disclosed, e.g., in U.S. Patent No. 4,784,950 (wild-type factor VII). In some embodiments, the factor VII polypeptide is human factor VIIa. In one series of embodiments, the factor VII polypeptides are factor VII-related polypeptides that exhibits at least about 10%, preferably at least about 30%, more preferably at least about 50%, and most preferably at least about 70%, of the specific biological activity of human factor VIIa. In some embodiments, the factor VII polypeptides have an amino acid sequence that differs from the sequence of wild-type factor VII by insertion, deletion, or substitution of one or more amino acids.

Non-limiting examples of factor VII variants having substantially the same or improved biological activity as wild-type factor VII include S52A-FVII, S60A-FVII (Iino et al., Arch. Biochem. Biophys. 352: 182-192, 1998); L305V-FVII, L305V/M306D/D309S-FVII, L305I-FVII, L305T-FVII, F374P-FVII, V158T/M298Q-FVII, V158D/E296V/M298Q-FVII, K337A-FVII, M298Q-FVII, V158D/M298Q-FVII, L305V/K337A-FVII, V158D/E296V/M298Q/L305V-FVII, V158D/E296V/M298Q/K337A-FVII, V158D/E296V/M298Q/L305V/K337A-FVII, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII; FVIIa variants exhibiting increased proteolytic stability as disclosed in U.S. Patent No. 5,580,560; factor VIIa that has been proteolytically cleaved between residues 290 and 291 or between residues 315 and 316 (Møllerup et al., Biotechnol. Bioeng. 48:501-505, 1995); and oxidized forms of factor VIIa (Kornfelt et al., Arch. Biochem. Biophys. 363:43-54, 1999). Non-limiting examples of factor VII variants having substantially reduced or modified biological activity relative to wild-type factor VII include R152E-FVIIa (Wildgoose et al., Biochem 29:3413-3420, 1990), S344A-FVIIa (Kazama et al., J. Biol. Chem. 270:66-72, 1995), FFR-FVIIa (Holst et al., Eur. J. Vasc. Endovasc. Surg. 15:515-520, 1998), and factor VIIa lacking the Gla domain, (Nicolaisen et al., FEBS Letts. 317:245-249, 1993). Non-limiting examples of chemically modified factor VII polypeptides and sequence variants are described, e.g., in U.S. Patent No. 5,997,864.

The biological activity of factor VIIa in blood clotting derives from its ability to (i) bind to tissue factor (TF) and (ii) catalyze the proteolytic cleavage of Factor IX or Factor X to produce activated Factor IX or X (Factor IXa or Xa, respectively).

For purposes of the invention, biological activity of factor VII polypeptides ("factor VII biological activity") may be quantified by measuring the ability of a preparation to promote blood clotting using factor VII-deficient plasma and thromboplastin, as described, e.g., in U.S. Patent No. 5,997,864. In this assay, biological activity is expressed as the reduction in clotting time relative to a control sample and is converted to "factor VII units" by comparison with a pooled human serum standard containing 1 unit/ml factor VII activity. Alternatively, factor VIIa biological activity may be quantified by

- (i) Measuring the ability of factor VIIa or a factor VIIa -related polypeptide to produce activated Factor X (Factor Xa) in a system comprising TF embedded in a lipid membrane and Factor X. (Persson et al., J. Biol. Chem. 272:19919-19924, 1997);
- (ii) Measuring Factor X hydrolysis in an aqueous system ("In Vitro Proteolysis Assay", see below);
- (iii) Measuring the physical binding of factor VIIa or a factor VIIa -related polypeptide to TF using an instrument based on surface plasmon resonance (Persson, FEBS Letts. 413:359-363, 1997); and
- (iv) Measuring hydrolysis of a synthetic substrate by factor VIIa and/or a factor VIIa -related polypeptide ("In Vitro Hydrolysis Assay", see below); and
- (v) Measuring generation of thrombin in a TF-independent in vitro system.

The term "factor VII biological activity" or "factor VII activity" is intended to include the ability to generate thrombin; the term also includes the ability to generate thrombin on the surface of activated platelets in the absence of tissue factor.

A factor VIIa preparation that may be used according to the invention is, without limitation, NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark).

#### Factor IX polypeptides:

The present invention encompasses factor IX polypeptides, such as, e.g., those having the amino acid sequence disclosed in, e.g., Jaye et al., Nucleic Acids Res. 11: 2325-2335, 1983. (wild-type human factor IX).

In practicing the present invention, any factor IX polypeptide may be used that is effective in preventing or treating bleeding. This includes factor IX polypeptides derived from blood or plasma, or produced by recombinant means.

As used herein, "factor IX polypeptide" encompasses, without limitation, factor IX, as well as factor IX-related polypeptides. The term "factor IX" is intended to encompass, without limitation, polypeptides having the amino acid sequence as described in Jaye et al., Nucleic Acids Res. 1983 (see above) (wild-type human factor IX), as well as wild-type Factor IX derived from other species, such as, e.g., bovine, porcine, canine, murine, and salmon Factor IX. It further

encompasses natural allelic variations of Factor IX that may exist and occur from one individual to another. Also, degree and location of glycosylation or other post-translation modifications may vary depending on the chosen host cells and the nature of the host cellular environment. The term "Factor IX" is also intended to encompass Factor IX polypeptides in their uncleaved (zymogen) form, as well as those that have been proteolytically processed to yield their  
5        respective bioactive forms, which may be designated Factor IXa.

"Factor IX-related polypeptides" include, without limitation, factor IX polypeptides that have either been chemically modified relative to human factor IX and/or contain one or more amino acid sequence alterations relative to human factor IX (i.e., factor IX variants), and/or  
10       contain truncated amino acid sequences relative to human factor IX (i.e., factor IX fragments). Such factor IX-related polypeptides may exhibit different properties relative to human factor IX, including stability, phospholipid binding, altered specific activity, and the like. The term "factor IX-related polypeptides" are intended to encompass such polypeptides in their uncleaved (zymogen) form, as well as those that have been proteolytically processed to yield  
15       their respective bioactive forms, which may be designated "factor IXa-related polypeptides" or "activated factor IX-related polypeptides".

As used herein, "factor IX-related polypeptides" encompasses, without limitation, polypeptides exhibiting substantially the same or improved biological activity relative to wild-type human factor IX, as well as polypeptides, in which the factor IX biological activity has been  
20       substantially modified or reduced relative to the activity of wild-type human factor IX. These polypeptides include, without limitation, factor IX or factor IXa that has been chemically modified and factor IX variants into which specific amino acid sequence alterations have been introduced that modify or disrupt the bioactivity of the polypeptide.

It further encompasses polypeptides with a slightly modified amino acid sequence, for  
25       instance, polypeptides having a modified N-terminal end including N-terminal amino acid deletions or additions, and/or polypeptides that have been chemically modified relative to human factor IX.

Factor IX-related polypeptides, including variants of factor IX, whether exhibiting substantially the same or better bioactivity than wild-type factor IX, or, alternatively, exhibiting  
30       substantially modified or reduced bioactivity relative to wild-type factor IX, include, without limitation, polypeptides having an amino acid sequence that differs from the sequence of wild-type factor IX by insertion, deletion, or substitution of one or more amino acids.

Factor IX-related polypeptides, including variants, encompass those that exhibit at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at  
35       least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, and at least about 130%, of the specific activity of wild-type factor IX that has been produced in the same cell type, when tested in the factor IX activity assay as described in the present specification.

Factor IX-related polypeptides, including variants, having substantially the same or improved biological activity relative to wild-type factor IX encompass those that exhibit at least about 25%, preferably at least about 50%, more preferably at least about 75%, more preferably at least about 100%, more preferably at least about 110%, more preferably at least about 120%,  
5 and most preferably at least about 130% of the specific biological activity of wild-type human factor IX that has been produced in the same cell type when tested in one or more of the specific factor IX activity assays as described. For purposes of the invention, factor IX biological activity may be quantified as described later in the present description ("assay part").

Factor IX-related polypeptides, including variants, having substantially reduced  
10 biological activity relative to wild-type factor IX are those that exhibit less than about 25%, preferably less than about 10%, more preferably less than about 5% and most preferably less than about 1% of the specific activity of wild-type factor IX that has been produced in the same cell type when tested in one or more of the specific factor IX activity assays as described above.

Non-limiting examples of factor IX polypeptides include plasma-derived human factor IX  
15 as described, e.g., in Chandra et al., *Biochem. Biophys. Acta* 1973, 328:456; Andersson et al., *Thromb. Res.* 1975, 7:451; Suomela et al., *Eur. J. Biochem.* 1976, 71:145.

In some embodiments the factor IX are factor IX-related polypeptides wherein the ratio between the activity of said factor IX polypeptide and the activity of native human factor IX (wild-type factor IX) is at least about 1.25 when tested in the "chromogenic assay" (see below);  
20 in other embodiments, the ratio is at least about 2.0; in further embodiments, the ratio is at least about 4.0.

Commercially available FIX products (so-called replacement products) are derived from normal pooled plasma or genetically engineered mammalian cell lines. Replacement products are often classified according to final purity, defined as specific activity (international units of clotting factor activity per mg of protein, IU/mg). Intermediate products have relatively low specific activity (< 50 IU/mg) because they also contain extraneous plasma proteins, such as fibrinogen, fibronectin and other non-coagulant proteins. High purity (>50 IU/mg) and ultra high purity (> 160 IU/mg) contain little or virtual no other plasma proteins other than albumin added as a stabiliser.

30 Non-limiting examples of commercially available Factor IX products (concentrates and preparations) that may be used according to the present invention are, for example, without limitation, BeneFix (rFIX) from AHP/Genetics Institute, Mononine (FIX) from Aventis, Proplex (FIX complex) from Baxter, Bebulin VH (FIX complex) from Baxter, FACTEUR IX-LFB (human plasma-based FIX) both from Laboratoire Francais du Fractionnement et des Biotechnologies (LFB), Immunine (Baxter/Immuno), Octanyne (Octapharma), Octanine F (Octapharma), Mono FIX-VF (CSL),  
35 Novact M (Kaketsuken)

Non-limiting examples of high and ultra high activity products are Immunine (Baxter/Immuno), Octanyne (Octapharma), Octanine F (Octapharma), Mono FIX-VF (CSL) (all high); BeneFix (AHP/Genetics Institute), Mononine (Aventis), Novact M (Kaketsuken) (all ultra high).

5 **Definitions:**

In the present context the three-letter or one-letter indications of the amino acids have been used in their conventional meaning as indicated in table 1. Unless indicated explicitly, the amino acids mentioned herein are L-amino acids. It is to be understood, that the first letter in, for example, K337 represent the amino acid naturally present at the indicated position wild-type factor VII, and that, for example, K337A-FVIIa designates the FVII-variant wherein the amino acid represented by the one-letter code K naturally present in the indicated position is replaced by the amino acid represented by the one-letter code A.

Table 1: Abbreviations for amino acids:

Amino acid	Tree-letter code	One-letter code
Glycine	Gly	G
Proline	Pro	P
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Cysteine	Cys	C
Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Histidine	His	H
Lysine	Lys	K
Arginine	Arg	R
Glutamine	Gln	Q
Asparagine	Asn	N
Glutamic Acid	Glu	E
Aspartic Acid	Asp	D

15

The terms "factor VII", "Factor VII" or "FVII" may be used interchangeably. The terms "factor VIIa", "Factor VIIa" or "FVIIa" may be used interchangeably. The terms "factor IX" or "Factor IX" or "FIX" may be used interchangeably.

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In this context, "subjects with an impaired thrombin generation" means subjects who cannot generate a full thrombin burst on the activated platelet surface and includes subjects having a generation of thrombin less than the thrombin-generation in subjects having a fully functioning, normal haemostatic system, including a normal amount and function of coagulation factors, platelets and fibrinogen, and includes, without limitations, subjects lacking factor IX; subjects with a lowered number of platelets or platelets with a defective function (e.g., thrombocytopenia or thrombasthenia Glanzmann or subjects with excessive bleeds); subjects having lowered levels of

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prothrombin, FX or FVII; subjects having a lowered level of several coagulation factors (e.g., due to excessive bleeding as a consequence of trauma or extensive surgery); and subjects with lowered plasma concentrations of fibrinogen (e.g., multitransfused subjects).

The term "enhancement of the haemostatic system" means an enhancement of the ability to generate thrombin. The term "enhancing haemostasis" is intended to encompass the situations when the measured thrombin generation for a test sample containing a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is prolonged relative to the individual thrombin generation of a control sample containing only the factor VII or factor VII-related polypeptide or the factor IX or factor IX-related polypeptide, respectively, when tested in the same thrombin generation assay. The thrombin generation may be assayed as described in the thrombin generation assay of the present description (see "assay part").

Clot lysis time, clot strength, fibrin clot formation, and clotting time are clinical parameters used for assaying the status of patient's haemostatic system. Blood samples are drawn from the patient at suitable intervals and one or more of the parameters are assayed by means of, e.g., thromboelastography as described by, e.g., Meh et al., Blood Coagulation & Fibrinolysis 2001;12:627-637; Vig et al., Hematology, Vol. 6 (3) pp. 205-213 (2001); Vig et al., Blood coagulation & fibrinolysis, Vol. 12 (7) pp. 555-561 (2001) Oct; Glidden et al., Clinical and applied thrombosis/hemostasis, Vol. 6 (4) pp. 226-233 (2000) Oct; McKenzie et al., Cardiology, Vol. 92 (4) pp. 240-247 (1999) Apr; or Davis et al., Journal of the American Society of Nephrology, Vol. 6 (4) pp. 1250-1255 (1995).

The term "prolonging clot lysis time" is intended to encompass the situations when the measured clot lysis time for a test sample containing a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is prolonged relative to the individual clot lysis time of a control sample containing only the factor VII or factor VII-related polypeptide or the factor IX or factor IX-related polypeptide, respectively, when tested in the same clot lysis assay. The clot lysis time may be assayed as described above.

The term "increasing clot strength" is intended to encompass the situations when the measured clot strength, e.g., mechanical strength, for a test sample containing a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is increased relative to the individual clot lysis time of a control sample containing only the factor VII or factor VII-related polypeptide or the factor IX or factor IX-related polypeptide, respectively, when tested in the same clot strength assay. The clot strength may be assayed as described, e.g. in Carr et al, 1991. (Carr ME, Zekert SL. Measurement of platelet-mediated force development during plasma clot formation. AM J MED SCI 1991; 302: 13-8), or as described above by means of thromboelastography.

The term "enhancing fibrin clot formation" is intended to encompass the situations when the measured rate for or degree of fibrin clot formation for a test sample containing a

preparation of a factor VII or factor VII-related polypeptide and a preparation of a preparation of a factor IX or factor IX-related polypeptide is increased relative to the individual rate for or degree of fibrin clot formation of a control sample containing only the factor VII or factor VII-related polypeptide or the factor IX or factor IX-related polypeptide, respectively, when tested in the same clotting assay. The fibrin clot formation may be assayed as described above.

The term "shortening clotting time" is intended to encompass the situations when the measured time for clot formation (clotting time) for a test sample containing a preparation of a factor VII or factor VII-related polypeptide and a preparation of a preparation of a factor IX or factor IX-related polypeptide is increased relative to the individual clotting time of a control sample containing only the factor VII or factor VII-related polypeptide or the factor IX or factor IX-related polypeptide respectively, when tested in the same clotting assay. The clotting time may be assayed by means of standard PT og aPTT assays, which are known to the general skilled person.

As used herein the term "bleeding disorder" reflects any defect, congenital, acquired or induced, of cellular or molecular origin that is manifested in bleeding episodes. Examples of bleeding disorders include, but are not limited to, clotting factor deficiencies (e.g. deficiency of coagulation factors VIII, IX, XI or VII), clotting factor inhibitors, defective platelet function (e.g., Glanzmann thrombasthenia and Bernard-Soulier syndrome), thrombocytopenia, von Willebrand's disease, and coagulopathy such as that caused by a dilution of coagulation proteins, increased fibrinolysis and lowered number of platelets due to bleedings and/or transfusions (e.g., in multi transfused subjects having been subjected to surgery or trauma).

Bleeding refers to extravasation of blood from any component of the circulatory system. The term "bleeding episodes" is meant to include unwanted, uncontrolled and often excessive bleeding in connection with surgery, trauma, or other forms of tissue damage, as well as unwanted bleedings in subjects having bleeding disorders. Bleeding episodes may occur in subjects having a basically normal coagulation system but experiencing a (temporary) coagulopathy, as well as in subjects having congenital or acquired coagulation or bleeding disorders. In subjects having a defective platelet function, the bleedings may be likened to bleedings caused by haemophilia because the haemostatic system, as in haemophilia, lacks or has abnormal essential clotting "compounds" (e.g., platelets or von Willebrand factor protein). In subjects who experience extensive tissue damage, for example in association with surgery or vast trauma, the normal haemostatic mechanism may be overwhelmed by the demand of immediate haemostasis and they may develop excessive bleeding in spite of a basically (pre-trauma or pre-surgery) normal haemostatic mechanism. Such subjects, who further often are multi transfused, develop a (temporary) coagulopathy as a result of the bleeding and/or transfusions (i.e., a dilution of coagulation proteins, increased fibrinolysis and lowered number of platelets due to the bleeding and/or transfusions). Bleedings may also occur in organs such as the brain, inner ear region and eyes; these are areas with limited possibilities for surgical haemostasis and thus problems with achiev-

ing satisfactory haemostasis. Similar problems may arise in the process of taking biopsies from various organs (liver, lung, tumour tissue, gastrointestinal tract) as well as in laparoscopic surgery and radical retropubic prostatectomy. Common for all these situations is the difficulty to provide haemostasis by surgical techniques (sutures, clips, etc.) which also is the case when bleeding is diffuse (e.g., haemorrhagic gastritis and profuse uterine bleeding). Bleedings may also occur in subjects on anticoagulant therapy in whom a defective haemostasis has been induced by the therapy given; these bleedings are often acute and profuse. Anticoagulant therapy is often given to prevent thromboembolic disease. Such therapy may include heparin, other forms of proteoglycans, warfarin or other forms of vitamin K-antagonists as well as aspirin and other platelet aggregation inhibitors, such as, e.g., antibodies or other inhibitors of GP IIb/IIIa activity. Bleeding episodes are also meant to include, without limitation, uncontrolled and excessive bleeding in connection with surgery or trauma in subjects having acute haemarthroses (bleedings in joints), chronic haemophilic arthropathy, haematomas, (e.g., muscular, retroperitoneal, sublingual and retropharyngeal), bleedings in other tissue, haematuria (bleeding from the renal tract), cerebral haemorrhage, surgery (e.g., hepatectomy), dental extraction, and gastrointestinal bleedings (e.g., UGI bleeds). The bleeding episodes may be associated with inhibitors against factor VIII; haemophilia A; haemophilia A with inhibitors; haemophilia B; deficiency of factor VII; deficiency of Factor XI; thrombocytopenia; deficiency of von Willebrand factor (von Willebrand's disease); severe tissue damage; severe trauma; surgery; laparoscopic surgery; haemorrhagic gastritis; taking biopsies; anticoagulant therapy; upper gastroentestinal bleedings (UGI); or stem cell transplantation. The bleeding episodes may be profuse uterine bleeding; occurring in organs with a limited possibility for mechanical haemostasis; occurring in the brain; occurring in the inner ear region; or occurring in the eyes. The terms "bleeding episodes" and "bleedings" may, where appropriate, be used interchangeably.

In this context, the term "treatment" is meant to include both prevention of an expected bleeding, such as, for example, in surgery, and regulation of an already occurring bleeding, such as, for example, in trauma, with the purpose of inhibiting or minimising the bleeding. The above-referenced "expected bleeding" may be a bleeding expected to occur in a particular tissue or organ, or it may be an unspecified bleeding. Prophylactic administration of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is thus included in the term "treatment".

The term "subject" as used herein is intended to mean any animal, in particular mammals, such as humans, and may, where appropriate, be used interchangeably with the term "patient".

The factor VII or factor VII-related polypeptides and factor IX or factor IX-related polypeptides as defined in the present specification may be administered simultaneously or sequentially. The factors may be supplied in single-dosage form wherein the single-dosage form contains both coagulation factors, or in the form of a kit-of-parts comprising a preparation of a factor VII or factor VII-related polypeptide as a first unit dosage form and a preparation of a factor

IX or factor IX-related polypeptide as a second unit dosage form. The second unit dosage form may be in the form of a high-, medium- or low-activity factor IX product. High-activity products are preferred. Most preferred are recombinant high-activity products. Whenever a first or second or third, etc., unit dose is mentioned throughout this specification this does not indicate the preferred order of administration, but is merely done for convenience purposes

By "simultaneous" dosing of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide is meant administration of the coagulation factor proteins in single-dosage form, or administration of a first coagulation factor protein followed by administration of a second coagulation factor protein with a time separation of no more than 15 minutes, preferably 10, more preferred 5, more preferred 2 minutes. Either factor may be administered first.

By "sequential" dosing is meant administration of a first coagulation factor protein followed by administration of a second coagulation factor protein with a time separation of up to 2 hours, preferably from 1 to 2 hours, more preferred up to 1 hour, more preferred from 30 minutes to 1 hour, more preferred up to 30 minutes, more preferred from 15 to 30 minutes. Either of the two unit dosage form, or coagulation factor proteins, may be administered first. Preferably, both products are injected through the same intravenous access.

By "level of factor IX" or "factor IX level" is meant the level of the patient's clotting factor IX activity compared to the level in healthy subjects. The level is designated as a percentage of the normal level. The terms may, where appropriate, be used interchangeably.

By "reduced level of factor IX" or "reduced factor IX level" is meant a decrease in the presence or activity of Factor IX in the blood stream compared to the mean factor IX level in a population of subjects having no coagulation factor IX deficiency or inhibitors to coagulation factor IX. Based on its purification from human plasma, the concentration of factor IX in the normal adult is about 300- 400 microg/ml of plasma.

In normal healthy individuals, factor IX activity and antigen levels vary between 50 and 160 % of normal pooled plasma. Clinically, the level of circulating factor IX can be measured by either a coagulant or an immunologic assay. Factor IX procoagulant activity is determined by the ability of the patient's plasma to correct the clotting time of factor IX-deficient plasma (e.g., in an APTT assay, see below; see also "assay part" of the present description).

One unit of factor IX has been defined as the amount of factor IX present in one millilitre of normal (pooled) human plasma (corresponding to a factor IX level of 100 %).

One unit of factor VII is defined as the amount of factor VII present in 1 ml of normal (pooled) plasma, corresponding to about 0.5 µg protein. After activation 50 units correspond to about 1 µg protein.

By "deficiency" is meant a decrease in the presence or activity of, e.g., factor IX in plasma compared to that of normal healthy individuals. The term may, where appropriate, be used interchangeably with "reduced factor IX level".

By "APTT" or "aPTT" is meant the activated partial thromboplastin time (described by, e.g., Proctor RR, Rapaport SI: The partial thromboplastin time with kaolin; a simple screening test for first-stage plasma clotting factor deficiencies. Am J Clin Pathol 36:212, 1961).

5 By "factor IX-responsive syndrome" is meant a syndrome where exogenous factor IX administered to the subject in need thereof may prevent, cure or ameliorate any symptoms, conditions or diseases, expected or present, caused by the syndrome. Included are, without limitation, syndromes caused by a reduced level of factor IX, e.g., bleeding disorders such as, without limitation, haemophilia B, or syndromes caused by inhibitors to factor IX.

10 By "factor VII-responsive syndrome" is meant a syndrome where exogenous factor VII, preferably factor VIIa, administered to the subject in need thereof may prevent, cure or ameliorate any symptoms, conditions or diseases, expected or present, caused by the syndrome. Included are, without limitation, syndromes caused by a reduced level of clotting factors VIII, IX, XI or VII, clotting factor inhibitors, defective platelet function (e.g., Glanzmann thrombasthenia and Bernard-Soulier syndrome), thrombocytopenia, von Willebrand's disease, and coagulopathy  
15 such as that caused by a dilution of coagulation proteins, increased fibrinolysis and lowered number of platelets due to bleedings and/or transfusions (e.g., in multi transfused subjects having been subjected to surgery or trauma).

"Half-life" refers to the time required for the plasma concentration of a factor VII or factor VII-related polypeptide or a factor IX or factor IX-related polypeptide to decrease from a  
20 particular value to half of that value.

By "primary haemostasis" is meant the initial generation of thrombin by FXa and TF:factor VIIa, the subsequent activation of platelets and formation of the initial loose plug of activated, adhered platelets which has not yet been stabilized by fibrin and, finally, by cross-linked fibrin. If not stabilized by the fibrin formed during the second step of the haemostatic  
25 process (maintained haemostasis), the plug is easily dissolved by the fibrinolytic system.

By "secondary haemostasis" or "maintained haemostasis" is meant the secondary, full, and major, burst or generation of thrombin taking place on the surface of activated platelets and catalysed by factor IXa and factor IXa, the subsequent formation of fibrin and the stabilization of the initial platelet plug. Stabilization of the plug by fibrin leads to full haemostasis.

30 By "full haemostasis" is meant the formation of a stable and solid fibrin clot or plug at the site of injury which effectively stops the bleeding and which is not readily dissolved by the fibrinolytic system. In this context, the term haemostasis will be used to represent full haemostasis as described above.

As used herein, a "preparation" of a coagulation factor, e.g., factor IX, is one in which  
35 factor IX is the predominant factor. In one embodiment the coagulation factor is present in the preparation in an amount of more than 20% (w/w) of the total amount of protein, more preferred 30%, more preferred 40%, more preferred 50%, more preferred 60%, more preferred

70%, more preferred 80%, more preferred 90%, more preferred 95%, more preferred 98%, more preferred 99%.

In a preferred embodiment, the coagulation factor is present in an amount of more than 50% (w/w) of the total amount of coagulation factor protein, more preferred 80%, more preferred 90%, more preferred 95%, more preferred 98%, more preferred 99%.

The total amount of protein in such preparation may be measured by generally known methods, e.g. by measuring optical density. Amounts of factor IX coagulation protein may be measured by generally known methods such as standard Elisa immuno assays. In general terms, such assay is conducted by contacting a solution of the factor IX protein-containing preparation with an anti-FIX antibody immobilised onto the elisa plate, subsequently contacting the immobilised antibody-factor IX complex with a second anti-FIX antibody carrying a marker, the amounts of which, in a third step, are measured. The amounts of each coagulation factor may be measured in a similar way using appropriate antibodies. The total amount of coagulation factor protein present in a preparation is determined by adding the amounts of the individual coagulation factor proteins. In one embodiment, the preparation comprises isolated coagulation factor. In another embodiment the preparation is free of coagulation factor II and coagulation factor IIa.

As used herein, the term "isolated" refers to coagulation factors, e.g., factor IX or factor IX-related polypeptides that have been separated from the cell in which they were synthesized or the medium in which they are found in nature (e.g., plasma or blood). Separation of polypeptides from their cell of origin may be achieved by any method known in the art, including, without limitation, removal of cell culture medium containing the desired product from an adherent cell culture; centrifugation or filtration to remove non-adherent cells; and the like. Separation of polypeptides from the medium in which they naturally occur may be achieved by any method known in the art, including, without limitation, affinity chromatography, such as, e.g., on an anti-Factor VII or anti-factor IX antibody column, respectively; hydrophobic interaction chromatography; ion-exchange chromatography; size exclusion chromatography; electrophoretic procedures (e.g., preparative isoelectric focusing (IEF)), differential solubility (e.g., ammonium sulfate precipitation), or extraction and the like.

#### **Abbreviations:**

TF	tissue factor
FVII	factor VII in its single-chain, unactivated form
FVIIa	factor VII in its activated form
rFVIIa	recombinant factor VII in its activated form
factor IX	factor IX in its zymogenic, unactivated form
factor IXa	factor IX in its activated form
rfactor IX	recombinant factor IX
rfactor IXa	recombinant factor IXa

**Preparation of compounds:**

Human purified Factor VIIa suitable for use in the present invention is preferably made by DNA recombinant technology, e.g. as described by Hagen et al., Proc.Natl.Acad.Sci. USA 83: 2412-2416, 1986, or as described in European Patent No. 200.421 (ZymoGenetics, Inc.).

Factor VII may also be produced by the methods described by Broze and Majerus, J.Biol.Chem. 255 (4): 1242-1247, 1980 and Hedner and Kiesel, J.Clin.Invest. 71: 1836-1841, 1983. These methods yield Factor VII without detectable amounts of other blood coagulation factors. An even further purified Factor VII preparation may be obtained by including an additional gel filtration as the final purification step. factor VII is then converted into activated factor VIIa by known means, e.g. by several different plasma proteins, such as factor XIIa, IX a or Xa. Alternatively, as described by Bjoern et al. (Research Disclosure, 269 September 1986, pp. 564-565), factor VII may be activated by passing it through an ion-exchange chromatography column, such as Mono Q® (Pharmacia fine Chemicals) or the like.

Factor VII -related polypeptides may produced by modification of wild-type Factor VII or by recombinant technology. Factor VII -related polypeptides with altered amino acid sequence when compared to wild-type Factor VII may be produced by modifying the nucleic acid sequence encoding wild-type factor VII either by altering the amino acid codons or by removal of some of the amino acid codons in the nucleic acid encoding the natural factor VII by known means, e.g. by site-specific mutagenesis.

It will be apparent to those skilled in the art that substitutions can be made outside the regions critical to the function of the factor VIIa or factor IX-molecule and still result in an active polypeptide. Amino acid residues essential to the activity of the Factor VII or factor VII-related polypeptide or factor IX or factor IX-related polypeptide, and therefore preferably not subject to substitution, may be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (see, e.g., Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, mutations are introduced at every positively charged residue in the molecule, and the resultant mutant molecules are tested for coagulant, respectively cross-linking activity to identify amino acid residues that are critical to the activity of the molecule. Sites of substrate-enzyme interaction can also be determined by analysis of the three-dimensional structure as determined by such techniques as nuclear magnetic resonance analysis, crystallography or photoaffinity labelling (see, e.g., de Vos et al., 1992, *Science* 255: 306-312; Smith et al., 1992, *Journal of Molecular Biology* 224: 899-904; Wlodaver et al., 1992, *FEBS Letters* 309: 59-64).

The introduction of a mutation into the nucleic acid sequence to exchange one nucleotide for another nucleotide may be accomplished by site-directed mutagenesis using any of the methods known in the art. Particularly useful is the procedure that utilizes a super coiled, double stranded DNA vector with an insert of interest and two synthetic primers containing the de-

sired mutation. The oligonucleotide primers, each complementary to opposite strands of the vector, extend during temperature cycling by means of *Pfu* DNA polymerase. On incorporation of the primers, a mutated plasmid containing staggered nicks is generated. Following temperature cycling, the product is treated with *DpnI*, which is specific for methylated and hemi-methylated DNA to digest the parental DNA template and to select for mutation-containing synthesized DNA. Other procedures known in the art for creating, identifying and isolating variants may also be used, such as, for example, gene shuffling or phage display techniques.

Separation of polypeptides from their cell of origin may be achieved by any method known in the art, including, without limitation, removal of cell culture medium containing the desired product from an adherent cell culture; centrifugation or filtration to remove non-adherent cells; and the like.

Optionally, Factor VII or factor VII-related polypeptides may be further purified. Purification may be achieved using any method known in the art, including, without limitation, affinity chromatography, such as, e.g., on an anti-Factor VII antibody column (see, e.g., Wakabayashi et al., J. Biol. Chem. 261:11097, 1986; and Thim et al., Biochem. 27:7785, 1988); hydrophobic interaction chromatography; ion-exchange chromatography; size exclusion chromatography; electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction and the like. See, generally, Scopes, Protein Purification, Springer-Verlag, New York, 1982; and Protein Purification, J.C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989. Following purification, the preparation preferably contains less than about 10% by weight, more preferably less than about 5% and most preferably less than about 1%, of non-Factor VII or factor VII-related polypeptides derived from the host cell.

Factor VII or factor VII-related polypeptides may be activated by proteolytic cleavage, using Factor XIIIa or other proteases having trypsin-like specificity, such as, e.g., Factor IXa, kallikrein, Factor Xa, and thrombin. See, e.g., Osterud et al., Biochem. 11:2853 (1972); Thomas, U.S. Patent No. 4,456,591; and Hedner et al., J. Clin. Invest. 71:1836 (1983). Alternatively, Factor VII or factor VII-related polypeptides may be activated by passing it through an ion-exchange chromatography column, such as Mono Q® (Pharmacia) or the like. The resulting activated Factor VII or factor VII-related polypeptide may then be formulated and administered as described below.

Factor IX for use within the present invention may be isolated from plasma according to known methods, such as those disclosed, e.g., by Chandra et al., Biochem. Biophys. Acta 1973, 328:456; Andersson et al., Thromb. Res. 1975, 7:451; Suomela et al., Eur. J. Biochem. 1976, 71:145. It is preferred, however, to use recombinant factor IX so as to avoid to the use of blood- or tissue-derived products that carry a risk of disease transmission. Human purified Factor IX suitable for use in the present invention is preferably made by DNA recombinant technology, e.g. as described in EP 107278 (British Technology Group), US 5171569 (Nat. res. Dev. Corp.), EP 430930 (British Technology Group).



Factor IX-related polypeptides may produced by modification of wild-type factor IX or by recombinant technology. Factor IX-related polypeptides with altered amino acid sequence when compared to wild-type factor IX may be produced by modifying the nucleic acid sequence encoding wild-type factor IX either by altering the amino acid codons or by removal of some of the amino acid codons in the nucleic acid encoding the natural factor IX by known means, e.g. by site-specific mutagenesis, as described in more detail above. Separation of polypeptides from their cell of origin may be achieved by any method known in the art, including, without limitation, removal of cell culture medium containing the desired product from an adherent cell culture; centrifugation or filtration to remove non-adherent cells; and the like. Optionally, the factor IX or factor IX-related polypeptides may be further purified. Purification may be achieved using any method known in the art, including, without limitation, affinity chromatography, such as, e.g., on an anti-factor IX antibody column; hydrophobic interaction chromatography; ion-exchange chromatography (e.g. as described in US 6034222; size exclusion chromatography; electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction and the like, as described in more detail above. Following purification, the preparation preferably contains less than about 10% by weight, more preferably less than about 5% and most preferably less than about 1%, of non-factor IX or factor IX-related polypeptides derived from the host cell. The resulting activated factor IX or factor IX-related polypeptide may then be formulated and administered as described below.

As will be appreciated by those skilled in the art, it is preferred to use factor IX polypeptides and factor VII polypeptides syngeneic with the subject in order to reduce the risk of inducing an immune response. Preparation and characterization of non-human factor IX has been disclosed by, e.g., Fujikawa et al., Biochemistry 1973, 12:4938 (bovine FIX) The present invention also encompasses the use of such factor IX polypeptides and factor VII polypeptides within veterinary procedures.

#### ***Pharmaceutical Compositions and Methods of Use***

The preparations of the present invention may be used to treat any factor IX responsive syndrome, such as, e.g., bleeding disorders, including, without limitation, those caused by clotting factor deficiencies (e.g., haemophilia B), or by (low or medium titre of) inhibitors to factor IX.

The preparations of the present invention may be used to treat any factor VII responsive syndrome, such as, e.g., bleeding disorders, including, without limitation, syndromes caused by a reduced level of clotting factors VIII, IX, XI or VII, clotting factor inhibitors, defective platelet function (e.g., Glanzmann thrombasthenia and Bernard-Soulier syndrome), thrombocytopenia, von Willebrand's disease, and coagulopathy such as that caused by a dilution of coagulation proteins, increased fibrinolysis and lowered number of platelets due to bleedings and/or transfusions (e.g., in multi transfused subjects having been subjected to surgery or trauma).

Pharmaceutical compositions comprising a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide according to the present invention are primarily intended for parenteral administration for prophylactic and/or therapeutic treatment. Preferably, the pharmaceutical compositions are administered  
5 parenterally, i.e., intravenously, subcutaneously, or intramuscularly; intravenously being most preferred. They may also be administered by continuous or pulsatile infusion.

Pharmaceutical compositions or formulations according to the invention comprise a preparation of a preparation of a factor VII or factor VII-related polypeptide, or a preparation of a preparation of a factor IX or factor IX-related polypeptide, or a preparation of a preparation of a  
10 factor VII or factor VII-related polypeptide in combination with a preparation of a preparation of a factor IX or factor IX-related polypeptide in combination with, preferably dissolved in, a pharmaceutically acceptable carrier, preferably an aqueous carrier or diluent. A variety of aqueous carriers may be used, such as water, buffered water, 0.4% saline, 0.3% glycine and the like. The preparations of the invention can also be formulated using non-aqueous carriers, such  
15 as, e.g., in the form of a gel or as liposome preparations for delivery or targeting to the sites of injury. Liposome preparations are generally described in, e.g., U.S. Patents Nos. 4,837,028, 4,501,728, and 4,975,282. The compositions may be sterilised by conventional, well-known sterilisation techniques. The resulting aqueous solutions may be packaged for use or filtered under aseptic conditions and lyophilised, the lyophilised preparation being combined with a  
20 sterile aqueous solution prior to administration.

The compositions may contain pharmaceutically acceptable auxiliary substances or adjuvants, including, without limitation, pH adjusting and buffering agents and/or tonicity adjusting agents, such as, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc.

25 Formulations may further include one or more diluents, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, controlled release, etc. One skilled in this art may formulate the compositions of the invention an appropriate manner, and in accordance with accepted practices, such as those disclosed in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton, PA, 1990.  
30 Thus, a typical pharmaceutical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution and 10 mg of the preparation.

The compositions containing the preparations of the present invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a subject already suffering from a disease, as described above,  
35 in an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be

understood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix.

Local delivery of the preparations of the present invention, such as, for example, topical application, may be carried out, e.g., by means of a spray, perfusion, double balloon catheters, stent, incorporated into vascular grafts or stents, hydrogels used to coat balloon catheters, or other well established methods. In any event, the pharmaceutical compositions should provide a quantity of the preparation sufficient to effectively treat the condition.

The concentration of factor VII or factor VII-related polypeptide, factor IX or factor IX-related polypeptide, or factor VII or factor VII-related polypeptide in combination with factor IX or factor IX-related polypeptide in these formulations can vary widely, i.e., from less than about 0.5% by weight, usually at or at least about 1% by weight to as much as 15 or 20% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. Administration by injection or infusion, in particular injection, is preferred. Thus, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are prepared in a form suitable for intravenous administration, such as a preparation that is either a dissolved lyophilized powder or a liquid formulation containing both the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide in one dosage form, or a dissolved lyophilized powder or a liquid formulation containing the factor VII or factor VII-related polypeptide in one dosage form and dissolved lyophilized powder or a liquid formulation containing the factor IX or factor IX-related polypeptide in another dosage form.

It is to be understood that the amount of factor VII or factor VII-related polypeptide and the amount of factor IX or factor IX-related polypeptide together comprise an aggregate effective amount for treating the bleeding episode.

It must be kept in mind that the materials of the present invention may generally be employed in serious disease or injury states, that is, life threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and general lack of immunogenicity of factor VIIa and factor IX in humans, it is possible and may be felt desirable by the treating physician to administer a substantial excess of these compositions.

In prophylactic applications, compositions containing a preparation of a factor VII or factor VII-related polypeptide and a preparation of a factor IX or factor IX-related polypeptide are administered to a subject susceptible to or otherwise at risk of a disease state or injury to enhance the subject's own coagulative capability. Such an amount is defined to be a "prophylactically effective dose." It is to be understood that the amount of factor VII or factor VII-related polypeptide and the amount of factor IX or factor IX-related polypeptide together comprise an aggregate effective amount for preventing a bleeding episode.

Single or multiple administrations of the compositions can be carried out with dose levels and patterns being selected by the treating physician. The compositions may be

administered one or more times per day or week. An effective amount of such a pharmaceutical composition is the amount that provides a clinically significant effect against bleeding episodes. Such amounts will depend, in part, on the particular condition to be treated, age, weight, and general health of the subject, and other factors evident to those skilled in the art.

5           The composition of the invention is generally administered in a single dose before the expected bleeding or at the start of the bleeding. It may however also be given repeatedly (in multiple doses) preferably with intervals of 2-4-6-12 hour, depending on the dose given and the condition of the subject.

10           For treatment in connection with deliberate interventions, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide will typically be administered within about 24 hours prior to performing the intervention, and for as much as 7 days or more thereafter. Administration as a coagulant can be by a variety of routes as described herein.

15           The composition may be in the form of a single preparation (single-dosage form) comprising both a preparation of a preparation of a factor VII or factor VII-related polypeptide and a preparation of a preparation of a factor IX or factor IX-related polypeptide in suitable concentrations. The composition may also be in the form of a kit-of-parts consisting of a first unit dosage form comprising a preparation of a preparation of a factor VII or factor VII-related polypeptide and a second unit dosage form comprising a preparation of a preparation of a factor IX or factor IX-related polypeptide. In this case, the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide should be administered one after the other, preferably within about 15 minutes of each other, for example within 10 minutes of each other or, preferably, within 5 minutes or, more preferred, within 2 minutes of each other. Either of the two unit dosage forms can be administered first.

25           The kit includes at least two separate pharmaceutical compositions. The kit includes container means for containing the separate compositions such as a divided bottle or a divided foil packet. Typically the kit includes directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms, are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

30           The amount of factor VII or factor VII-related polypeptide and the amount of factor IX or factor IX-related polypeptide administered according to the present invention may vary from a ratio of between about 1:100 to about 100:1 (w/w). The ratio of factor VII to factor IX may thus be, e.g., about 1:100, or 1:90, or 1:80, or 1:70 or 1:60, or 1:50, or 1:40, or 1:30, or 1:20, or 1:10, or 1:5, or 1:2, or 1:1, or 2:1, or 5:1, or 10:1, or 20:1, or 30:1, or 40:1, or 50:1, or 60:1, or 70:1, or 80:1, or 90:1, or 100:1; or between about 1:90 to about 1:1, or between about 1:80 to about 1:2, or between about 1:70 to about 1:5, or between about 1:60 to about 1:10, or between about 1:50 to about 1:25, or between about 1:40 to about 1:30, or between about 90:1 to about 1:1, or between about 80:1 to

35

about 2:1, or between about 70:1 to about 5:1, or between about 60:1 to about 10:1, or between about 50:1 to about 25:1, or between about 40:1 to about 30:1.

The dose of the Factor VII or factor VII-related polypeptide ranges from what corresponds to about 0.05 mg to about 500 mg/day of wild-type Factor VII, e.g., from about 1 mg  
5 to about 200 mg/day, or, e.g., from about 5 mg to about 175 mg/day for a 70-kg subject as loading and maintenance doses, depending on the weight of the subject, the condition and the severity of the condition.

The dose of the factor IX or factor IX-related polypeptide ranges from what corresponds to about 0.01 mg to about 500 mg/day of wild-type factor IX, e.g., from about 1 mg to  
10 about 200 mg/day, or, e.g., from about 5 mg to about 175 mg/day for a 70-kg subject as loading and maintenance doses, depending on the weight of the subject, the condition and the severity of the condition.

When treating subjects with a reduced level of factor IX, the below doses are preferred:  
15 When dosing of a factor IX or factor IX-related polypeptide to a plasma activity level up to 10% of normal Factor IX activity:

Preferred Factor VII or factor VII-related polypeptide levels: 15 – 300 microgram/kg b.w.

More preferred Factor VII or factor VII-related polypeptide levels: 30 – 250 microgram/kg b.w.

Most preferred Factor VII or factor VII-related polypeptide levels: 60 – 180 microgram/kg b.w.

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When dosing of a factor IX or factor IX-related polypeptide to a plasma activity level up to 30% of normal Factor IX activity:

Preferred Factor VII or factor VII-related polypeptide levels: 15 – 300 microgram/kg b.w.

More preferred Factor VII or factor VII-related polypeptide levels: 30 – 250 microgram/kg b.w.

25 Most preferred Factor VII or factor VII-related polypeptide levels: 60 – 180 microgram/kg b.w.

When dosing of a factor IX or factor IX-related polypeptide to a plasma activity level up to 50 % of normal Factor IX activity:

Preferred Factor VII or factor VII-related polypeptide levels: 10 – 300 microgram/kg b.w.

30 More preferred Factor VII or factor VII-related polypeptide levels: 20 – 200 microgram/kg b.w.

Most preferred Factor VII or factor VII-related polypeptide levels: 40 – 140 microgram/kg b.w.

When dosing of a factor IX or factor IX-related polypeptide to a plasma activity level up to 80 % of normal Factor IX activity:

35 Preferred Factor VII or factor VII-related polypeptide levels: 5 – 300 microgram/kg b.w.

More preferred Factor VII or factor VII-related polypeptide levels: 10 – 180 microgram/kg b.w.

Most preferred Factor VII or factor VII-related polypeptide levels: 60 – 120 microgram/kg b.w.

When dosing of a factor IX or factor IX-related polypeptide to a plasma activity level up to 100 % of normal Factor IX activity:

Preferred Factor VII or factor VII-related polypeptide levels: 5 – 300 microgram/kg b.w.

5 More preferred Factor VII or factor VII-related polypeptide levels: 10 – 180 microgram/kg b.w.

More preferred Factor VII or factor VII-related polypeptide levels: 30 – 120 microgram/kg b.w.

Most preferred Factor VII or factor VII-related polypeptide levels: 60 – 120 microgram/kg b.w.

10 However, a maximal a factor IX or factor IX-related polypeptide dose of 80% of normal factor IX activity will rarely be exceeded, as higher substitution levels have been associated with increased incidence of thrombotic complications.

Dosing can be calculated by assuming that 1 unit per kg of b.w. of FIX replacement raises the plasma activity by approx. 0.01 U per ml (1%). The patient's factor IX level is monitored by drawing blood samples at suitable intervals and analysing for factor IX activity (see specifica-  
15 tion above).

#### Assays:

##### Test for factor VIIa activity:

A suitable assay for testing for factor VIIa activity and thereby selecting suitable factor  
20 VIIa variants can be performed as a simple preliminary *in vitro* test:

##### In Vitro Hydrolysis Assay

Native (wild-type) factor VIIa and factor VIIa variant (both hereafter referred to as "factor VIIa") may be assayed for specific activities. They may also be assayed in parallel to directly compare their specific activities. The assay is carried out in a microtiter plate (MaxiSorp, Nunc,  
25 Denmark). The chromogenic substrate D-Ile-Pro-Arg-p-nitroanilide (S-2288, Chromogenix, Sweden), final concentration 1 mM, is added to factor VIIa (final concentration 100 nM) in 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM CaCl<sub>2</sub> and 1 mg/ml bovine serum albumin. The absorbance at 405 nm is measured continuously in a SpectraMax™ 340 plate reader (Molecular De-  
vices, USA). The absorbance developed during a 20-minute incubation, after subtraction of the  
30 absorbance in a blank well containing no enzyme, is used to calculate the ratio between the activities of variant and wild-type factor VIIa:

$$\text{Ratio} = (A_{405 \text{ nm}} \text{ factor VIIa variant}) / (A_{405 \text{ nm}} \text{ factor VIIa wild-type}).$$

Based thereon, factor VIIa variants with an activity comparable to or higher than native factor VIIa may be identified, such as, for example, variants where the ratio between the activity of the  
35 variant and the activity of native factor VII (wild-type FVII) is around, versus above 1.0.

The activity of factor VIIa or factor VIIa variants may also be measured using a physiological substrate such as factor X, suitably at a concentration of 100-1000 nM, where the factor

Xa generated is measured after the addition of a suitable chromogenic substrate (eg. S-2765). In addition, the activity assay may be run at physiological temperature.

In Vitro Proteolysis Assay

5 Native (wild-type) Factor VIIa and Factor VIIa variant (both hereafter referred to as "Factor VIIa") are assayed in parallel to directly compare their specific activities. The assay is carried out in a microtiter plate (MaxiSorp, Nunc, Denmark). Factor VIIa (10 nM) and Factor X (0.8 microM) in 100 microL 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM CaCl<sub>2</sub> and 1 mg/ml bovine serum albumin, are incubated for 15 min. Factor X cleavage is then stopped by the addition of 50 microL 50  
10 mM Hepes, pH 7.4, containing 0.1 M NaCl, 20 mM EDTA and 1 mg/ml bovine serum albumin. The amount of Factor Xa generated is measured by addition of the chromogenic substrate Z-D-Arg-Gly-Arg-p-nitroanilide (S-2765, Chromogenix, Sweden), final concentration 0.5 mM. The absorbance at 405 nm is measured continuously in a SpectraMax™ 340 plate reader (Molecular Devices, USA). The absorbance developed during 10 minutes, after subtraction of the absorbance in a blank well containing no FVIIa, is used to calculate the ratio between the proteolytic activities of variant and wild-  
15 type Factor VIIa:

$$\text{Ratio} = (\text{A}_{405 \text{ nm}} \text{ Factor VIIa variant}) / (\text{A}_{405 \text{ nm}} \text{ Factor VIIa wild-type}).$$

Based thereon, factor VIIa variants with an activity comparable to or higher than native factor VIIa may be identified, such as, for example, variants where the ratio between the activity of the  
20 variant and the activity of native factor VII (wild-type FVII) is around, versus above 1.0.

Thrombin generation assay:

The ability of factor VII or factor VII-related polypeptides (e.g., variants) or factor IX or factor IX-related polypeptides (e.g., variants) to generate thrombin can be measured in an assay  
25 comprising all relevant coagulation factors and inhibitors at physiological concentrations and activated platelets (as described on p. 543 in Monroe et al. (1997) Brit. J. Haematol. 99, 542-547 which is hereby incorporated as reference).

Test for factor IX activity:

30 Suitable assays for testing for factor IX activity, and thereby providing means for selecting suitable factor IX variants for use in the present invention, can be performed as simple *in vitro* tests as described, for example, in Wagenvoort et al., Haemostasis 1990;20(5):276-88 ("the chromogenic assay")

Factor IX biological activity may also be quantified by measuring the ability of a  
35 preparation to correct the clotting time of factor IX-deficient plasma, e.g., as described in Nilsson et al., 1959.(Nilsson IM, Blombaeck M, Thilen A, von Francken I., Carriers of haemophilia A - A laboratory study, Acta Med Scan 1959; 165:357). In this assay, biological activity is expressed as units/ml plasma (1 unit corresponds to the amount of FIX present in normal pooled plasma.

Aspects of the invention:

5           In one aspect, the invention concerns a pharmaceutical composition comprising a FVII polypeptide and a FIX polypeptide as the sole active coagulation factors. In one embodiment, the FVII polypeptide is human recombinant FVIIa. In one embodiment, the FIX polypeptide is human recombinant FIX. In one embodiment, the FVII polypeptide and the FIX polypeptide are mixed. In one embodiment, the FVII polypeptide and the FIX polypeptide are in separate containers. In one embodiment, the composition is for home treatment.

10           In another aspect, the invention concerns a kit for treatment of bleeding episodes comprising

- a) An effective amount of a FVII polypeptide and, optionally, a pharmaceutically acceptable carrier in a first unit dosage form;
- 15       b) An effective amount of a FIX polypeptide and, optionally, a pharmaceutically acceptable carrier in a second unit dosage form; and
- c) Container means for containing said first and second dosage forms.

          In one embodiment, the FVII polypeptide is human recombinant FVIIa. In one embodiment, the FIX polypeptide is human recombinant FIX. In one embodiment, the kit is for home treatment.

20           In another aspect, the invention concerns the use of a FVII polypeptide and a FIX polypeptide for the preparation of a medicament for the treatment of bleedings in a subject suffering from a FIX responsive syndrome. In another aspect, the invention concerns the use of a FVII polypeptide and a FIX polypeptide for the preparation of a medicament for the treatment of bleedings in a subject having a reduced level of FIX. In one embodiment, the medicament is for treatment of

25           bleeding episodes in haemophilia A patients. In one embodiment, the medicament comprises a mixture of a FVII polypeptide and a FIX polypeptide. In one embodiment, the medicament is prepared in the form of a first dosage form comprising a FVII polypeptide and a second dosage form comprising a FIX polypeptide. In one embodiment, the FVII polypeptide is human recombinant FVIIa. In one embodiment, the FIX polypeptide is human recombinant FIX.

30           In another aspect, the invention concerns a method to enhance haemostasis in a subject suffering from a FIX responsive syndrome compared to when the subject is treated with FIX as the only coagulation protein, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide

          In another aspect, the invention concerns a method to enhance haemostasis in a subject

35           having a reduced level of FIX compared to when the subject is treated with FIX as the only coagulation protein, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.



In another aspect, the invention concerns a method to enhance formation of thrombin in a subject suffering from a FIX responsive syndrome compared to when the subject is treated with FIX as the only coagulation protein, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method to enhance formation of thrombin in a subject having a reduced level of FIX compared to when the subject is treated with FIX as the only coagulation protein, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method for reducing the number of administrations of coagulation factor protein needed to accomplish haemostasis in a subject suffering from a FIX responsive syndrome compared to the number of administrations needed when FIX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method for reducing the number of administrations of coagulation factor protein needed to accomplish haemostasis in a subject having a reduced level of FIX compared to the number of administrations needed when FIX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method for reducing the amount of administered coagulation factor protein needed to accomplish haemostasis in a subject suffering from a FIX responsive syndrome compared to the amount of administered coagulation factor protein needed when FIX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method for reducing the amount of administered coagulation factor protein needed to accomplish haemostasis in a subject having a reduced level of FIX compared to the amount of administered coagulation factor protein needed when FIX is administered to the subject as the only coagulation factor protein, the method comprising administering to a subject in need thereof an effective amount of a FVII polypeptide and an effective amount of a FIX polypeptide.

In another aspect, the invention concerns a method of treating bleedings in a subject suffering from a FIX responsive syndrome, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and a FIX polypeptide.

In another aspect, the invention concerns a method of treating bleedings in a subject having a reduced level of FIX, the method comprising administering to the subject in need thereof an effective amount of a FVII polypeptide and a FIX polypeptide.

In one embodiment of the methods, the FVII polypeptide is human recombinant FVIIa.

5 In one embodiment, the FIX polypeptide is human recombinant FIX. In one embodiment, the the subject suffers from haemophilia B.

10 The present invention is further illustrated by the following examples, which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

## EXAMPLES

**Example 1: *In vivo treatment of a haemophilia B patient with intracranial bleeds***

When a non-inhibitor haemophilia B patient suffering from intracranial bleeds is treated with a commercially available FIX product he will generally need between 8 and 16 injections or infusions of FIX to achieve haemostasis. The FIX infusion will intend to achieve an initial  
5 FIX plasma concentration of at least 80 % of normal level followed by a plasma concentration of 50 % for one week.

Such patient is treated with one dose of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a simultaneously administered FIX product, or with one dose  
10 of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a FIX product within a time separation, e.g., 5 minutes. Both products are injected through the same intravenous access. The patient experiences a reduced time to obtain bleeding arrest and a reduced number of injections to maintain haemostasis. This regiment leads to a reduced total amount of coagulation factor protein usage for bleeding arrest and haemostasis.

**Example 2: *In vivo treatment of a patient with chronic liver disease with diffuse upper gastrointestinal bleeds***

The patient is suffering from diffuse gastric bleeds due to haemorrhagic gastritis of unknown ethiology. The patient has reduced amounts of vitamin K dependent coagulation factors, especially factors VII and IX due to decreased liver function secondary to chronic hepatitis C. The  
20 patient has been transfused with red blood cells, fluids for i.v. injection, and fresh frozen plasma which contains coagulation factor IX.

Such patient is treated with one dose of 90-120 µg/kg b.w. of FVIIa and a simultaneously administered FFP product, or with one dose of 90-120 µg/kg b.w., FVIIa and a FFP product  
25 within a time separation, e.g., 5 minutes. Both products are injected through the same intravenous access. The patient experiences a reduced time to obtain bleeding arrest from multiple bleeding sites in his stomach and a reduced number of injections to maintain haemostasis. This regiment leads to a reduced total amount of coagulation factor protein usage for bleeding arrest and haemostasis and to improve survival.

**Example 3: *A non-inhibitor haemophilia B patient suffering a muscular bleed in the arm with symptoms of a compartment syndrome***

The patient is a non-inhibitor haemophilia B patient suffering from a major traumatic muscular bleed in the right arm with symptoms of a compartment syndrome.

When such a patient is treated with a commercially available FIX product he will generally need between 8 and 16 injections or infusions of FIX to achieve haemostasis. The FIX infusion will intend to achieve an initial FIX plasma concentration of at least 80 % of normal level followed by a plasma concentration of 50 % for one week.

Such patient is treated with one dose of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a simultaneously administered FIX product, or with one dose of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a FIX product within a time separation, e.g., 5 minutes. Both products are injected through the same intravenous access.

The said combinations of NovoSeven and a FIX product provides improved effect on time to bleeding arrest, degree of peripheral nerve and vascular damage and the size and complexity of the surgical intervention compared to the effect of either NovoSeven or a FIX product administered alone.

**Example 4: Assaying coagulation status of a non-inhibitor haemophilia B patient**

The patient is a non-inhibitor haemophilia B patient suffering from bleeds, e.g., intracranial bleeds.

When such a patient is treated with a commercially available FIX product he will generally need between 8 and 16 injections or infusions of FIX to achieve haemostasis. The FIX infusion will intend to achieve an initial FIX plasma concentration of at least 80 % of normal level followed by a plasma concentration of 50 % for one week.

Such patient is treated with one dose of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a simultaneously administered FIX product, or with one dose of 90-180 µg/kg b.w. of NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) and a FIX product within a time separation, e.g., 5 minutes. Both products are injected through the same intravenous access. Ten minutes after administration of the latter of the two coagulation proteins a blood sample are drawn and a whole blood coagulation analysis performed using the thromboelastographic method which is a standardised assay, clinical relevant for coagulation status (see, for example, Meh et al., BLOOD COAGULATION & FIBRINOLYSIS 2001;12:627-637). Using standard parameter readings from such an assay enhanced fibrin clot formation; increased clot strength and prolonged clot lysis time are demonstrated. Such measurement in sequential blood samples demonstrates the variation of these parameters as function of time after injection of the factor VII and the factor IX products.

**Example 5: Shortening the Clotting Time with Combinations of factors VIIa and factor IX**

**METHODS:**

Clot assay: The specific clotting activity of recombinant human coagulation factor VIIa (rFVIIa), in the absence or presence of various concentrations of plasma purified human factor IX (FIX) was measured in one-stage assays as previously described (Persson et al., J Biol Chem 276: 29195-9, 2001). In short, aliquots (55 µl) of rFVIIa (0.2-3 µg/ml, Novo Nordisk stock) in 50 mM Pipes, 100 mM NaCl, 2 mM EDTA, 1% BSA, pH 7.2, were mixed with an equal volume buffer containing 50 mM CaCl<sub>2</sub> and phosphatidylcholine/phosphatidylserine vesicles (total phospholipid concentra-

tion 100  $\mu$ M; 80% phosphatidylcholine/20% phosphatidylserine), and clotting was started by adding 55  $\mu$ l FIX-deficient plasma (Helena Labs Helena Labs #5793) added various concentrations of FIX (10, 50, and 80 % of the plasma concentration, Haematologic Technologies). Clotting was followed for 500 seconds in an ACL 300 Research coagulometer (Instrumentation Laboratory, Milan, Italy) using the standard APTT program.

#### RESULTS:

Clot assay: rFVIIa and FIX, separately and in combination was added to FIX-deficient plasma and the clotting times was determined. Prior to addition of rFVIIa/FIX the clotting time of both plas-  
10 mas was longer than the 500 seconds monitoring time. The clot shortening effect of rFVIIa, in the absence and presence of FIX is shown in figure 1.

#### CONCLUSION:

These results demonstrate that the combination of rFVIIa and FIX is capable of shortening the  
15 clotting time of FIX-deficient plasma beyond what is seen when the proteins was added separately.

## CLAIMS

1. A pharmaceutical composition comprising a preparation of a factor VII or a factor VII-related polypeptide, and a preparation of a factor IX or a factor IX-related polypeptide.
- 5 2. A composition according to claim 1, wherein said factor VII or factor VII-related polypeptide is a factor VII-related polypeptide.
3. A composition according to claim 2, wherein said factor VII-related polypeptide is a factor VII  
10 amino acid sequence variant.
4. A composition according to claims 2 or claim 3, wherein the ratio between the activity of said factor VII-related polypeptide and the activity of native human factor VIIa (wild-type FVIIa) is at least about 1.25 when tested in the "In Vitro Hydrolysis Assay" as described in the present de-  
15 scription.
5. A composition according to claim 1, wherein said factor VII or factor VII-related polypeptide is a factor VII polypeptide.
- 20 6. A composition according to claim 5, wherein said factor VII is human factor VII
7. A composition according to claim 6, wherein said factor VII polypeptide is recombinant human factor VII.
- 25 8. A composition according to any one of claims 1 to 7, wherein said factor VII or factor VII-related polypeptide is in its activated form.
9. A composition according to claim 8, wherein said factor VII polypeptide is recombinant human factor VIIa.
- 30 10. A composition according to any one of claims 1-9, wherein said factor IX or factor IX-related polypeptide is a factor IX-related polypeptide.
11. A composition according to claim 10, wherein said factor IX-related polypeptide is a factor IX  
35 amino acid sequence variant.

12. A composition according to claim 10 or claim 11, wherein the ratio between the activity of said factor IX-related polypeptide and the activity of native human factor IX (wild-type FIX) is at least about 1.25 when tested in the "chromogenic assay" as described in the present description.
- 5 13. A composition according to any one of claims 1 to 9, wherein said factor IX or factor IX-related polypeptide is a factor IX polypeptide.
14. A composition according to claim 13, wherein said factor IX is human factor IX
- 10 15. A composition according to claim 14, wherein said factor IX polypeptide is recombinant human factor IX.
16. A composition according to any one of claims 1 to 15, wherein said factor VII or factor VII-related polypeptide and said factor IX or factor IX-related polypeptide are present in a ratio by mass of between about 100:1 and about 1:100 factor VII:factor IX
- 15 17. A kit of parts containing a treatment for bleeding episodes comprising
- a) An effective amount of a preparation of a factor VII or factor VII-related polypeptide and a pharmaceutically acceptable carrier in a first unit dosage form;
  - 20 b) An effective amount of a preparation of a factor IX or factor IX-related polypeptide and a pharmaceutically acceptable carrier in a second unit dosage form; and
  - c) Container means for containing said first and second dosage forms.
18. A kit according to claim 17, wherein said factor VII or factor VII-related polypeptide is a factor VII-related polypeptide.
- 25 19. A kit according to claim 18, wherein said factor VII-related polypeptides are factor VII amino acid sequence variants.
- 30 20. A kit according to claim 18 to 19, wherein the ratio between the activity of said factor VII-related polypeptide and the activity of native human factor VIIa (wild-type FVIIa) is at least about 1.25 when tested in the "In Vitro Hydrolysis Assay" as described in the present description.
- 35 21. A kit according to claim 17, wherein said factor VII or factor VII-related polypeptide is a factor VII polypeptide.
22. A kit according to claim 21, wherein said factor VII polypeptide is human factor VII

23. A kit according to claim 22, wherein said factor VII polypeptide is recombinant human factor VII.
24. A kit according to any one of claims 17 to 23, wherein said factor VII or factor VII-related polypeptide is in its activated form.
25. A kit according to claim 24, wherein said factor VII polypeptide is recombinant human factor VIIa.
26. A kit according to any one of claims 17-25, wherein said factor IX or factor IX-related polypeptide is a factor IX-related polypeptide.
27. A kit according to claim 26, wherein said factor IX-related polypeptide is a factor IX amino acid sequence variant.
28. A kit according to claim 26 or claim 27, wherein the ratio between the activity of said factor IX-related polypeptide and the activity of native human factor IX (wild-type FIX) is at least about 1.25 when tested in the "chromogenic assay" as described in the present description.
29. A kit according to any one of claims 17 to 25, wherein said factor IX or factor IX-related polypeptide is a factor IX polypeptide.
30. A kit according to claim 29, wherein said factor IX is human factor IX
31. A kit according to claim 30, wherein said factor IX polypeptide is recombinant human factor IX.
32. A kit according to any one of claims 17 to 31, wherein said factor VII or factor VII-related polypeptide and factor IX or factor IX-related polypeptide are present in a ratio by mass of between about 100:1 and about 1:100 factor VII:factor IX
33. Use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or a factor IX-related polypeptide for the manufacture of a medicament for treating bleeding episodes in a subject.
34. Use of a composition according to any one of claims 1 to 16, for the manufacture of a medicament for treating bleeding episodes in a subject.



35. Use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for reducing clotting time.

- 5     36. Use of a composition according to any one of claims 1 to 16, for the manufacture of a medicament for reducing clotting time.

37. Use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament  
10     for prolonging the clot lysis time.

38. Use of a composition according to any one of claims 1 to 16, for the manufacture of a medicament for prolonging the clot lysis time.

- 15     39. Use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for increasing clot strength.

40. Use of a composition according to any one of claims 1 to 16, for the manufacture of a medicament for increasing clot strength.  
20

41. Use of a preparation of a factor VII or factor VII-related polypeptide in combination with a preparation of a factor IX or factor IX-related polypeptide for the manufacture of a medicament for enhancing fibrin clot formation.  
25

42. Use of a composition according to any one of claims 1 to 16, for the manufacture of a medicament for enhancing fibrin clot formation.

43. Use according to any one of claims 33 to 42, wherein the subject is suffering from a factor IX responsive syndrome.  
30

44. Use according to any one of claims 33 to 43, wherein the subject, prior to treatment, has a reduced level of factor IX.

- 35     45. Use according to claim 43 or claim 44, wherein the subject is suffering from haemophilia A

46. Use according to any one of claims 33 to 45, wherein the medicament is in single-dosage form.

47. Use according to any one of claims 33 to 45, wherein the medicament is prepared in the form of a first dosage form comprising a preparation of a factor VII or factor VII-related polypeptide and a second dosage form comprising a preparation of a factor IX or factor IX-related polypeptide.
48. A method for treating bleeding episodes in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide, wherein the first and second amount together are effective to treat bleedings.
49. A method for reducing clotting time in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to reduce clotting time.
50. A method to enhance haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to enhance haemostasis.
51. A method for reducing the number of administrations of coagulation factor protein needed to arrest bleeding and maintain haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to arrest bleeding and maintain haemostasis.
52. A method for reducing the amount of administered coagulation factor protein needed to arrest bleeding and maintain haemostasis in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to arrest bleeding and maintain haemostasis.
53. A method for prolonging the clot lysis time in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related

polypeptide wherein the first and second amount together are effective to prolong the clot lysis time.

5 54. A method for increasing clot strength in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to increase clot strength.

10 55. A method for enhancing fibrin clot formation in a subject, the method comprising administering to a subject in need thereof a first amount of a preparation of a factor VII or factor VII-related polypeptide and a second amount of a preparation of a factor IX or factor IX-related polypeptide wherein the first and second amount together are effective to enhance fibrin clot formation.

15 56. A method according to any one of claims 48 to 55, wherein the subject is suffering from a factor IX responsive syndrome.

57. A method according to any one of claims 48 to 56, wherein the subject, prior to treatment, has a reduced level of factor IX.  
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58. A method according to claim 56 or claim 57, wherein the subject is suffering from haemophilia A

25 59. Method according to any one of claims 48 to 58, wherein the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered in single-dosage form.

30 60. Method according to any one of claims 48 to 58, wherein the factor VII or factor VII-related polypeptide and the factor IX or factor IX-related polypeptide are administered in the form of a first dosage form comprising a preparation of a factor VII or factor VII-related polypeptide and a second dosage form comprising a preparation of a factor IX or factor IX-related polypeptide.

61. Method according to claim 60, wherein the first dosage form and the second dosage form are administered with a time separation of no more than 15 minutes.

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62. A kit containing a treatment for bleeding episodes comprising

- d) An effective amount of a factor VII or factor VII-related polypeptide and an effective amount of a factor IX or factor IX-related polypeptide and a pharmaceutically acceptable carrier in a one-unit dosage form; and
- e) Container means for containing said one-unit dosage form.

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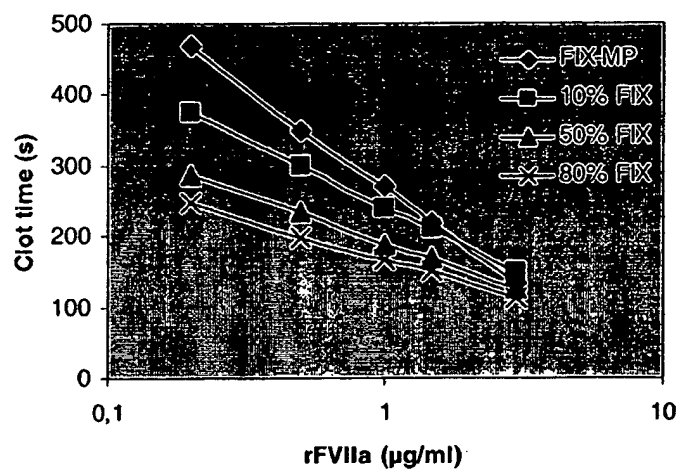


Figure 1

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 02/00080

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K38/36 A61P7/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, MEDLINE, PAJ, EMBASE, EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NITU I C ET AL: "Clinical experience with the use of clotting factor concentrates in oral anticoagulation reversal." CLIN. LAB. HAEM., vol. 20, 1998, pages 363-367, XP002902489 summary lines 4 and 5 and introduction. ---	1-47,62
X	WO 81 02105 A (BAXTER TRAVENOL LAB) 6 August 1981 (1981-08-06) abstract; claim 20 --- -/--	1-47,62

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

24 April 2002

Date of mailing of the international search report

19. 06. 2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Anna Björklund

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 02/00080

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1976 MANNUCCI P M ET AL: "CORRECTION OF ABNORMAL COAGULATION IN CHRONIC LIVER DISEASE BY COMBINED USE OF FRESH FROZEN PLASMA AND PROTHROMBIN COMPLEX CONCENTRATES" Database accession no. PREV197763018687 XP002902490 abstract &amp; LANCET, vol. 2, no. 7985, 1976, pages 542-545, ---</p>	1-47,62
X	<p>DR.CHUANSUMRIT A.: "Combined fresh frozen plasma with recombinant factor VIIa in restoring hemostasis for invasive procedures in children with liver diseases." THROMB HAEMOST , vol. 85, 2001, pages 748-749, XP002902491 the whole document -----</p>	1-47,62

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 02/00080

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 48-61  
because they relate to subject matter not required to be searched by this Authority, namely:  
See PCT Rule 39.1.(iv): Methods for treatment of the human or animal body by surgery or by therapy, as well as diagnostic methods.
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.2

Claims Nos.: 1-4, 8 , 10-12, 16-20,24, 26-28,32-33,35, 37, 39, 41, 47 and 62(partially)

Present claims 1-2,4,8,16-18,20,24,32-33,35,37,39,41,47 and 62 relate to an extremely large number of possible compounds, designated "factor VII-related polypeptides". Claims 1,10,12,16,28,32-33,35,37,39,41,47 and 62 relate to an extremely large number of possible compounds, designated "factor IX-related polypeptides". Furthermore, claims 3 and 19 and claims 11 and 27 relate to an extremely large number of possible variants, designated "factor VII amino acid sequence variants" and "factor IX amino acid sequence variants", respectively.

The claims contain so many possible polypeptides that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been limited and focused on the general aspect of combining the coagulation factors VII and IX, as well as on the invention as described in the examples of the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 02/00080

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8102105	A	06-08-1981	US 4287180 A 01-09-1981
			US 4286056 A 25-08-1981
			AT 9063 T 15-09-1984
			CA 1176563 A1 23-10-1984
			DE 3165600 D1 27-09-1984
			DK 427281 A 25-09-1981
			EP 0044343 A1 27-01-1982
			IT 1141963 B 08-10-1986
			JP 7145072 A 06-06-1995
			JP 7145073 A 06-06-1995
			JP 7145074 A 06-06-1995
			JP 7145075 A 06-06-1995
			JP 6086385 B 02-11-1994
			JP 57500382 A 04-03-1982
			WO 8102105 A1 06-08-1981
			US 4357321 A 02-11-1982
			US 4663164 A 05-05-1987
			ES 498902 D0 01-05-1982
			ES 8204601 A1 16-08-1982